PHYSIOLOGICAL RESPONSES OF ROSS 308 BROILER CHICKENS FED GRADED LEVELS OF MORINGA OLEIFERA LEAF MEAL (MOLM): SOME ASPECTS OF HAEMATOLOGY AND SERUM BIOCHEMISTRY

by

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Exact wording of the title of the thesis as appearing on the copies submitted for examination:

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I declare that the above thesis is my own work and that all the resources that I have used or quoted have been indicated and acknowledged by means of complete references.

_________________________ ___03/06/2019__________________

SIGNATURE DATE

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DEDICATION

I dedicate this whole work to the queen of my heart, a very strong yet humble beautiful woman I have ever known: my late Mother Mrs. Motsatsi Dorcas Modisaojang who taught me that I must always put education first as it is the only powerful weapon that no one can ever take away from me. I understand and know why you liked the late Nelson Mandela’s saying that reads, “It always seems impossible until it’s done”. I have made it against all odds and refused to give up. I had always finished the race like you taught me, and I promised you that I will always make you proud. I am what I am today because of you, how I wish you were still around to celebrate my achievements. Thanks for the love, and for believing in me Ma, I will forever cherish the moments I had with you, may your beautiful soul rest in perfect peace dear Mommy.

ABSTRACT

The high cost of feed materials and feed additives in developing nations has elicited interest in the search for sustainable alternatives. Moringa (*Moringa oleifera*), one of such sustainable alternatives is a tropical plant that has its usefulness investigated in this study. A 42-day study was designed to determine the response of Ross 308 broilers to dietary *Moringa oleifera* leaf meal supplementation. The *Moringa oleifera* leaves used for the study were analysed for proximate, mineral and composition as well as phytochemical contents before being incorporated in the diet. Day-old Ross 308 broiler chicks (n = 500) were allotted to five treatments in completely randomized design with each treatment replicated five times and each replicate having 20 chicks. The birds were subjected to diets supplemented with *Moringa oleifera* leaf meal at 0, 25, 50, 75 and 100 g/kg feed at both starter and finisher stage, respectively and designated as T1, T2, T3, T4 and T5. *Moringa oleifera* leaf meal level that supported optimum production and physiological variables was modelled using the quadratic function. At day 42, three birds per replicate were slaughtered to evaluate carcass and organ yields. Result of the proximate composition revealed that MOLM is rich in protein (32.37%) and neutral detergent fibre (52.16%). Mineral assay indicated that MOLM was high in calcium, sodium, potassium, sulphur and iron. Daily feed intake (FI), average daily gain (ADG) and feed conversion ratio were the same among the treatments with the exception of starter broilers on diet T1 that had higher ADG ($p<0.05$) than those on the other diets. Final live weight (FLW), mortality and gizzard weight were influenced ($p<0.05$) by *Moringa oleifera* leaf meal supplementation. *Moringa oleifera* leaf meal supplementation had no effect on parameters measured. *Moringa oleifera* leaf meal supplementation at 39.98 and 35.80 g/kg feed supported optimum FLW and ADG at starter phase and 46.88 g/kg feed MOLM supported optimum FLW at finisher phase. In conclusion, *Moringa oleifera* leaf meal is a good source of nutrients and suitable for production of enhanced cut parts in broiler chickens. Birds on 50 and 75 g *Moringa oleifera* leaf meal/kg feed had higher ($p<0.05$) packed cell volume (PCV), red blood cell (RBC) and glucose than those on the other 3 treatment diets. The white blood cell (WBC) counts for birds on 50 g *Moringa oleifera* leaf meal/kg feed were higher ($p<0.05$) than those on 100 g *Moringa oleifera* leaf meal/kg feed but similar ($p>0.05$) to those on 0, 25 and 75 g MOLM/kg feed. Blood platelet count maintained the trend 75 g > 0 g > 50 g > 100 g > 25 g MOLM/kg feed with birds on 75 g *Moringa oleifera* leaf meal/kg feed being statistically higher ($p<0.05$) than those on 25, 50 and 100 g MOLM/kg feed. Dietary *Moringa oleifera* leaf meal supplementation had no significant effect ($p>0.05$) on haemoglobin (Hb), total serum protein (TSP), albumin, cholesterol and uric acid. Triglyceride (TG) level of birds on 25, 75 and 100 g *Moringa oleifera* leaf meal/kg feed decreased significantly compared to those on 0 and 50 g MOLM/kg feed. Daily *Moringa oleifera* leaf meal supplementation had a significant effect ($p<0.05$) on the differential WBC count. Daily *Moringa oleifera* leaf meal supplementation with 26.99 g/kg feed and 31.95 g/kg feed respectively supported optimum PCV (38.62%) and glucose (245.42 mg/dl) in Ross 308 broilers. It is, therefore summarized that optimizing MOLM supplementation level in the ration of Ross 308 broilers could assist in improving their productivity.

Keywords: Moringa leaf, growth performance, blood characteristics, carcass and organ yields, optimization function, broiler chickens

TABLE OF CONTENT

LIST OF ABBREVIATIONS

AOAC: Association of Official Analytical Chemists

ADG: Average Daily Gain

ADF: Acid Detergent Fibre

AGDP: Agriculture's Gross Domestic Products

ANFs: Anti-nutritional Factors

ALT: Alanine Transaminase

AST: Aspartate Transaminase

BW: Breast weight

Ca: Calcium

Cu: Copper

Cr: Chromium

CF: Crude Fibre

CP: Crude Protein

CV: Coefficient of Variation

CW: Carcass weight

DM: Dry Matter

DoH: Department of Health

DFI: Daily Feed Intake

DP: Dressing percentage

DW: Drumstick weight

EAAs: Essential Amino Acids

EDTA: Ethylene-diamine Tetra Acetic acid

EE: Ether Extracts

FA: Fatty Acids

FAO: Food and Agriculture Organization of the United Nations:

FCR: Feed Conversion Ratio

Fe: Iron

FI: Feed Intake

FLW: Final Live Weight

GW: Gizzard weight

HDL-c: High Density cholesterol

HW: Heart weight

Hb: Haemoglobin

K: Potassium

LDL-c: Low-Density cholesterol

LW: Liver weight

LWs: Live Weights

MCV: Mean Cell Volume

MOLE: Moringa Oleifera Leaf Extract

MOLM: Moringa oleifera leaf meal

MOFM: Moringa Oleifera Forage Meal

Mn: Manganese

Mg: Magnesium

NA: Sodium

NDF: Neutral Detergent Fibre

NFE: Nitrogen-Free Extracts

NRC: Nutrient Requirement for poultry

P: Phosphorus

PCV: Packed Cell Volume

PUFA: Polyunsaturated Fatty Acids

RBC: Red Blood Cell

S: Sulphur

SBM: Soybean Meal

SD: Standard deviation

SEM: Standard Error of the Mean

SPSS: Statistical Software

TG: Triglyceride

TSP: Total Serum Protein

TPSD: The poultry site digital newsletter

TW: Thigh weight

UNISA: University of South Africa

WBC: White Blood Cell

WHO: World Health Organisation

Zn: Zinc

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Background

Poultry industry in developing countries is facing some challenges due to the rising cost of conventional energy and protein feedstuffs (Abbas, 2013). Chickens are the most important poultry species reared for generating income by the resource challenged rural households in the developing world. Most of the developing countries are situated in the tropics where the infrastructure is not well developed to produce the vital feed ingredients for livestock feeding (Melesse *et al*., 2013). Thus, the poultry industry has become an important economic activity in many countries, however, exposure to stressful conditions, diseases, deterioration of environmental conditions often result in serious economic losses (Kabir, 2009).

Banjo (2012) suggested that in the tropics some of the poultry problems as mentioned above are practically solved by paying attention to the areas of nutrient requirements of birds for maintenance and production, and the nutrient composition of the available feedstuffs. Although, with the increased competition for the traditional energy and protein feedstuff such as maize and soybean between man and animals, alternative nutrient sources for poultry feeding becomes a necessity and hence there is a need to look for cheap, locally available and less competitive substitute to some ingredients of poultry feeds and in particular protein sources (Gadzirai *et al*., 2012). As suggested by Banjo (2012), *Moringa oleifera* is one of the plants that can be utilized in this regard.

Moringa tree grows natural, it is drought resistant and easily accessible as they grow everywhere within the reach of most farmers. It is a perennial woody herb that can be easily adopted especially by poultry farmers. The tree is resistant to most pests and diseases, thus making it a cheap source of feed for animals (Gadzirai *et al*., 2012) and the leaves are the preferred part for use in animal diets as leaf meal because they are regarded as a non-conventional feedstuff, which could be of value for poultry feeding (Zanu *et al*., 2012). In addition, it is also important to ascertain the role of *Moringa oleifera* leaf meal supplementation on the production parameters of poultry. This will help to ascertain its effect on the physiological responses of the animals as well as ensure food security and socio-economic empowerment of the people.

1.2 PROBLEM STATEMENT

Maize and soybean have been traditionally used for livestock and poultry feed. The importance of maize and soybean as a human and industrial food ingredient coupled with drought in some parts of Africa has sometimes caused relative scarcity of these ingredients and an attendant increase in price invariably leading to an increase in the price of compounded feed (Olugbemi *et al.*, 2010). For example, in the present day reality, especially in South Africa with its attendant drought issues (a deficit of 25% is normally regarded as a severe meteorological drought but it can be safely assumed that a shortfall of 20% from normal rainfall will cause crop and water shortfalls in many regions accompanied by social and economic hardships), the use of soybean and maize in animal production becomes uneconomical and unsustainable. The prices of other conventional protein feed resources such as groundnut and fishmeal have recently become so expensive and this have made them uneconomical for use in compounding poultry feeds (Esonu *et al.*, 2001). Therefore, this call for a radical shift to search for a non-conventional feed resource that can act as a supplement that provide certain level of nutrient to reduce total dependence on the costly ingredients. One of such non-conventional feed resource available locally in South Africa is *Moringa oleifera* leaf plant. However, detailed study has not been carried out to ascertain the production potential of Ross boilers fed *Moringa oleifera* leaf meal supplemented diets. Production variables and carcass values have not been deeply investigated. Improvement of the production and carcass characteristics would enhance food security and economic empowerment of the rural people.

1.3 MOTIVATION

Data on nutrient profiles of *Moringa oleifera* leaf meal and productivity parameters of Ross 308 broiler chickens fed diets supplemented with *Moringa oleifera* leaf meal is fraught with inconsistencies, claims and counter claims. Aside from this, it is also very important to know the production indices following *Moringa oleifera* leaf meal supplementation in Ross 308 broilers as it will help in the formulation of diets to optimize the bird’s productivity. The benefit here is that the moringa tree grows natural in many areas of Limpopo Province of South Africa and no competition with humans have been observed, so it would be cheaper compared to soybean meal which has a direct competition for human dietary need. Thus, it is envisaged that knowledge of this production indices of Ross 308 broilers following *Moringa oleifera* leaf meal supplementation will help to improve the productivity of the bird and at the same time help to enhance the social and nutritional status of South African households and small-scale poultry farming.

1.4 AIM AND OBJECTIVES

1.4.1. AIM: The aim of the study was to evaluate the effects of dietary nutrient supplementation *Moringa oleifera* leaf meal on productivity, haematology and serum biochemistry physiology (blood and serum) of Ross 308 broiler chickens.

1.4.2. OBJECTIVES:

- To assess the proximate biochemical composition and mineral profiles of *Moringa oleifera* leaf meal (MOLM).

- To determine the growth performance indices of Ross 308 broiler chickens fed on diets containing various supplementation levels of MOLM leaf meal.

- To determine the impact of supplemental inclusion of dietary MOLM leaf meal on haematological and serum biochemical parameters of Ross 308 broiler chickens.

- To determine the effect of varying supplementation levels of dietary MOLM leaf meal on carcass and organ weight characteristics of Ross 308 broiler chickens.

- To determine the optimal supplementation level of MOLM leaf meal that significantly improved productive indices in Ross 308 broiler chickens using quadratic optimization model.

1.5 SIGNIFICANCE OF THE STUDY

Results from this study will help to indicate if there is any effect of supplemental MOLM on biochemical indices and immunity parameters (WBC & Lymphocytes) of Ross 308 broiler chickens and at the same time help to broaden the knowledge on dietary nutrient supplementation to improve productivity and health status of Ross 308 broiler chickens.

1.6 LIMITATIONS OF THE STUDY

The limitations on this study are minimal as it was funded by DST-IKS NRF at the University of Kwa Zulu Natal (Westville campus) in partnership with UNISA, and most of the required equipment's for the study were obtained locally.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The role of poultry meat in closing food insecurity gap in Africa as well as improving the socio-economic status of farm households cannot be neglected (Dieye *et al*., 2010). In South Africa, the poultry sector has been reported to contribute about 18% to Agriculture's Gross Domestic Products (TPSD, 2011). FAO data as cited in TPSD (2011) revealed that chicken meat production in Africa continent increased by about 2 million tonnes between 2000 and 2009 with South Africa (816.9 – 966.4 thousand tonnes) taking the lead, followed by Egypt (513.3–625.0 thousand tonnes) and Morocco (250.0–450.0 thousand tonnes). In South Africa, the demand for poultry meat has consistently increased over the years, and this pattern is expected to persist. This demand for poultry products is having a strong effect on the demand for feed and raw materials. It has become obvious that the demand for the conventional energy and protein concentrates is achievable. This has necessitated search for cheap and readily available local feed resources for use in feed formulations.

One of such local available feedstuffs is high in essential nutrients and beneficial bioactive compounds is *Moringa oleifera* leaf meal (Siddhuraju and Becker, 2003; Teixeira *et al*., 2014). Moringa, a drought resistant tree, is the most popular species among the 14 species in the family of Moringaceae. *Moringa oleifera* has its origin in India but grows well in the tropics and sub-tropics (Nsofor *et al*., 2012).

The use of Moringa in livestock ration is limited by the presence of anti-nutritional factors (Kachik *et al.*, 1992). However, the presence of these anti-nutritional agents in moringa products is removed via air drying, soaking or boiling in water (Ekpo and Eddy, 2005; Igwilo *et al*., 2007). The beneficial effect of moringa and its products in poultry nutrition have been documented (Olugbemi *et al*., 2010; Abou-Elezz *et al*., 2011).

However, the reported growth-promoting effect may be due to its direct nutritional and immune-stimulating actions of its phytochemicals (Fahey, 2005; Du *et al*., 2007; Ghazalah and Ali, 2008; Liaqat *et al*., 2016). The review aimed to bring the potential of moringa products to a global picture as a partial replacement for antibiotics and conventional protein concentrate in broiler chicken feed.

2.2 Chemical composition

2.21 Proximate analysis and mineral value

The *M. oleifera* root contains 6.33% ether extract (EE), 5.02% crude proteins (CP), 76.65% nitrogen-free extracts (NFE), 4.97% ash and 6.93% moisture (Igwilo *et al*., 2014). Alabi *et al.* (2017) observed that aqueous *M. oleifera* leaf extract (MOLE) is higher in CP (23.80%) than the root CP (Table 2.1). The disparity between the CP content of the two results could be attributed to the part of the plant used which is reported to influence nutrient content and also method of analysis can be one of disparity cause (Ustundag and Ozdogan, 2016). The CP of 23.80% obtained by Alabi *et al.* (2017) was lower than 29.55% reported by Nuhu (2010) for the leaf meal. Additionally, Nuhu (2010) reported that *M. oleifera* contain 2.23% EE, 19.25% crude fibre, 7.13% ash and 41.98% NFE. These findings are in agreement with Oduro *et al.* (2008) and Alabi et al. (2017) who observed that MOLM is a reservoir of nutrients (27.51% CP, 19.25% CF, 22.3% EE, 7.13% ash and 76.53% dry matter). The CP value of 23.80 to 29.55% reported for *M. oleifera* leaf meal (MOLM) by other authors (Oduro *et al*., 2008; Nuhu, 2010; Jiwuba *et al.* 2017; Alabi *et al.,* 2017) is within the CP value (20–33%) reported by Foidl and Paull (2008). In a similar nutritional study, Yusuf *et al*. (2018) observed that Moringa leaves is high in essential nutrients (4% moisture, 96% dry matter (DM), 31.5% CP, 0.4% EE, 35.5% neutral detergent fibre (NDF), 25.3% acid detergent fibre (ADF), and 9.7% total ash. *M. oleifera* leaf has high potassium and sodium content but low in calcium (Table 2.2), whereas the seeds have high potassium and sodium content (Tijani *et al*., 2015; Ustundag and Ozdogan, 2016). In comparison with other common leafy vegetables (cassava, amaranth, taro and pumpkin), moringa leaves are high in calcium, sodium and potassium content (Tijani *et al*., 2015).

Table 2.1. Proximate values of MOLM

Parameters [%]	1	2	3
Dry matter (DM)	94.25	90.65	93.60
Ether Extract (EE)	5.50	2.44	3.40
Crude protein (CP)	23.80	25.37	22.6
Crude fibre (CF)	16.57	17.41	10.10
Ash	9.75	5.89	7.80
Nitrogen free extracts (NFE)	38.63	-	49.60
Total sugars	-	39.02	-
Reducing sugars	-	13.62	-
Non reducing sugars	-	21.40	-

Adapted from 1 - Alabi *et al.* (2017); 2 - Alnidawi *et al.* (2016); 3 - Tijani *et al*. (2015)

Table 2.2. Mineral values moringa

Minerals	Leaves (mg/kg)*	Seeds (mg/ 100g)*	Leaf meal (mg/100g)**
Calcium (Ca)	0.4900	0.2925	6.98
Phosphorus (P)	0.3600	0.21600	-
Potassium (K)	1.3800	0.9615	7.09
Sodium (Na)	0.6700	0.7240	-
Magnesium (Mg)	0.2700	0.2998	-
Manganese (Mn)	0.0122	0.0125	-
Iron (Fe)	0.0415	0.0142	2.98
Zinc (Zn)	0.0047	0.0041	5.89
Copper (Cu)	0.0012	0.0006	-

Adapted from Tijani *et al.* (2015)**; Ustundag and Ozdogan (2016)

2.22 Amino acid composition

M. oleifera root is high in arginine and lysine while low in thiamine, riboflavin and histidine (Igwilo *et al*., 2014). Moringa leaf meal has all the 13 essential amino acids (EAAs) needed for normal physiological processes in poultry (Fuglie, 2001; Igwilo *et al.,* 2010). Moringa leaf and seed contain all essential amino acids (Table 2.3) but the leaf contains larger amount of isoleucine and smaller amount of tryptophan content (Chelliah *et al*., 2017). The EAA content of both the seeds and leaves is comparable to the reference values reported by the World Health Organisation. This is also in agreement with the values recorded by Foidl *et al*. (2001), Anhwange *et al*. (2004) and Sánchez-Machado *et al.* (2010) in the same leaf meal. A recent study by Chelliah *et al.* (2017) put glutamic acid as the most abundant EAA in MOLM and this finding was in harmony with the results of Nasab *et al*. (2016) in *Moringa peregrine*.

2.23 Vitamin composition

Fat- and water-soluble vitamins (retinol, tocopherol and ascorbic acid as well as carotenoids) are abundant in MOLM (DanMalam *et al*., 2001; Luqmans *et al*., 2012; Igwilo *et al*., 2014). Information exists that newly harvested moringa leaves are high in lutein and β-carotene (Liu *et al*., 2007; Saini *et al*., 2012). This result is in accordance with Saini *et al.,* (2013, 2014) who observed that lutein and β-caroteneare are abundant in *M. oleifera*. In comparison with other leaf meals used in animal nutrition, *M. oleifera* contains large amount of β- carotene (19.7 mg/100 g fresh weight) (Bhaskarachary *et al*., 1995). Carotenoids play a vital role in poultry nutrition because of its strong antioxidant property (Kuhnen *et al*., 2009; Nakazawa *et al*., 2009). Knowledge of the carotenoid value of M. oleifera is vital because of its role in pigmentation of egg yolk and broiler carcasses. An investigation by Chelliah *et al.* (2017) revealed that moringa leaves are high in vitamin E while seeds are high in vitamin C (Table 2.4). Also, the seeds are low in riboflavin and has no tocopherol (Chelliah *et al*., 2017).

Table 2.3. Amino Acid composition of *M. oleifera*

Amino acids [%]	Leaf	WHO	Seed pods	WHO
Arginine	3.70 - 4.30	1.88	9.44 - 11.00	8.06
Histidine	2.50 - 3.12	1.90	5.7 - 6.3	2.01
Isoleucine	5.90 - 9.10	2.33	7.7 - 8.3	4.35
Leucine	4.40 - 4.10	5.22	4.7 - 4.8	5.27
Lysine	3.30 - 4.60	3.60	3.7 - 4.2	3.24
Methionine	1.90 - 3.40	0.95	3.1 - 3.5	0.97
Phenylalanine	4.20 - 4.60	4.26	3.5 - 3.9	4.53
Tryptophan	1.50 - 1.90	2.10	3.7 - 4.3	2.30
Threonine	4.10 - 4.50	4.38	3.2 - 3.8	3.22
Valine	4.55 - 4.75	3.36	2.37 -3.36	3.09
Glycine	5.11- 5.13	5.15	4.7 - 5.0	5.00
Glutamate	15.33 -15.86	15.14	14.23 - 14.74	14.76
Serine	4.25 - 4.66	4.20	4.11 - 4.22	4.25
Alanine	3.23 - 3.90	3.43	3.55 - 4.29	3.23
Aspartate	6.44 - 6.86	6.86	6.02 -5.37	6.14

Adapted from Chelliah *et al.* (2017)

Table 2.4. Vitamin composition of *M. oleifera* [Adapted from Chelliah *et al.* (2017)]

Vitamins	Leaf	Seed
A [mg]	6.3 - 6.8	0.3 – 0.8
B_1 [mg]	2.59 - 2.64	0.05 – 0.06
B_2 [mg]	20.5-21.0	0.06 – 0.08
B_3 [mg]	8.2-9.6	1.2 – 1.9
B_5 [mg]	0.13-1.60	0.7 - 0.9
B_9 [µg]	39.5-40.0	46.0 – 48.0
C [mg]	17.3 – 19.4	124.0 - 130.0
E [mg]	113.0 – 121.0	-

2.3 Phytochemical composition

Phytochemical composition of *Moringa oleifera* plant has been documented as well as its role in ethno veterinary medicine (Isitua and Ibeh, 2013). According to Ogbe and Affiku (2011) and Alabi *et al.* (2017), *Moringa oleifera* is a storehouse of important bioactive compounds. Sharma and Paliwal (2013) have described saponin as one of the bioactive glycoside compounds that have a comprehensive medicinal and pharmacological actions such as anticoccidial, immune stimulant, antibacterial and anti-fungi effect. The use of saponin rich plant to reduce protozoa (Eimeria *species*) load in the digestive tract of poultry has been documented in the literature (Wina *et al.,* 2017). Studies by Alabi *et al.* (2017) have shown that leaf meal is higher in saponins than aqueous leaf extract (Fig. 2.1) and may serve as a novel plant for the development of new anticoccidial therapeutic drugs. Stohs and Hartman (2015) have reported that *Moringa oleifera* pods are abundant in phytochemical compounds that are of potential benefit to animal nutrition. Anwar *et al.* (2006) reported that *M. oleifera* contains simple sugar, rhamnose, glucosinolates and ibiocyanates. This finding is in harmony with Saini *et al.* (2016), who reported that *M. oleifera* is richly endowed with important biological active compounds such as glucosinolates, flavonoids and phenolic acids. The abundance of flavonoid confers the pharmacological activities of Moringa leaves (Mbikay, 2012; Ijarotimi *et al*., 2013).

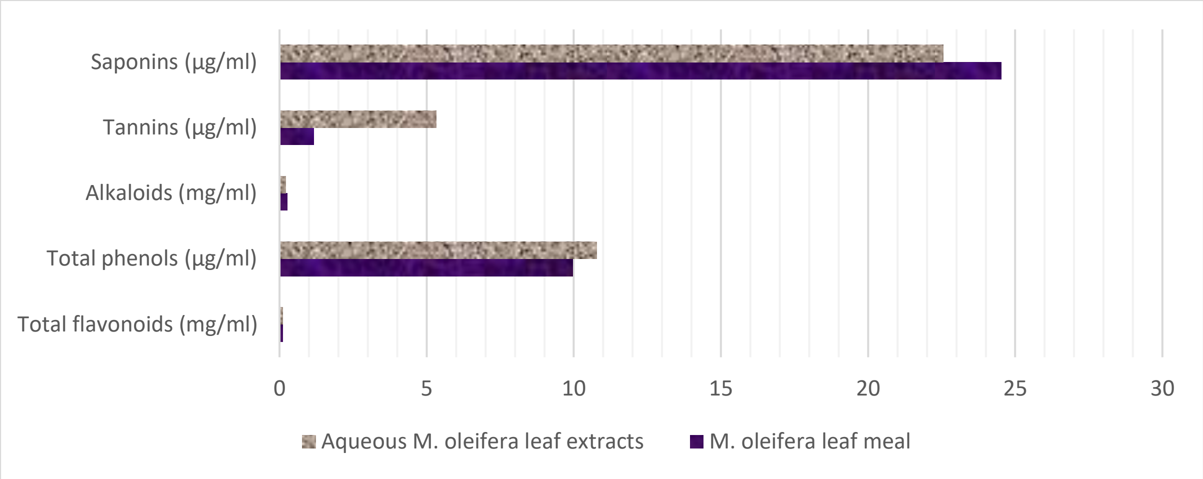

Fig. 2.1. Effect of presentation forms (water and powder) on phytochemical content of *M. oleifera* leaf. Adapted from Alabi *et al.* (2017).

2.4 Anti-nutrient factors

Anti-nutritional factors (ANFs) are biological compounds that are naturally present in human or animal feed, and their presence may reduce feed consumption or nutrient utilisation efficiency. Moringa leaves and seeds contain ANFs such as tannins, saponins and phytates. The harmful effect of phytates and oxalates in both animals and humans has been documented (Akubugwo *et al*., 2007). The major ANFs in *M. oleifera* seeds that may have harmful effect on broiler health and production are glucosinolates, haemagglutinins and alkaloids. *M. oleifera* roots were moderate in tannins and oxalates but low in saponins, phytates and cyanogenic glycosides (Igwilo *et al*., 2014). Tannins, a group of secondary metabolites, perform a vital part in plant defence against pathogenic microbes, herbivores and fluctuating weather conditions. Tannins form complexes with proteins in the digestive tract, thus making them unavailable for digestion and assimilation which will finally lead to protein deficiency syndrome (Igwilo *et al*., 2010). It has been observed that tannins, phytates and oxalates bind with calcium and zinc, hence reducing their availability for absorption in the gut (Akubugwo *et al*., 2007). It is worthy to note that these anti-nutritional factors contained in Moringa are easily detoxified by soaking in water or heat treatment (Ekpo and Eddy, 2005; Igwilo *et al.,* 2007). Soaking moringa seeds in water for 20–30 min have proved to be effective in removing the bitter taste in the seed kernel caused by alkaloids, saponins, cyanogens and glucosinolates (Igwilo *et al*., 2014). Unlike the seeds, the leaves are low in tannins (12 g/kg dry matter, DM) and phytates (21 g/kg DM) but have no trypsin inhibitors, amylase inhibitors, lectins, cyanogenic glucosides and glucosinolates.

2.5 Moringa and broiler performance

2.51 Broiler growth performance

The influence of forages on the digestive physiology and nutrient utilisation efficiency of broilers has been a subject of interest since it was observed that they influence the macroarchitecture of the gastro-intestinal tracts. Therefore, it has become pertinent to ascertain the efficiency of utilisation of novel feedstuffs with high dietary fibre such as moringa leaf meal on the performance of poultry. Feeding moringa forage meal–based diets improve fibre digestion and utilisation (Fig. 2.2) in broilers (Bustamante, 2014). It has been estimated that chickens are capable of utilising about 25% fibre present in the ration (Bertechini, 2006). The increased fibre digestibility on broilers on *M. oleifera* forage meal (MOFM) diet relative to the control birds is an indication that moringa leaf meal is high in digestible fibre. African and Cuban researchers have recommended 5–10% MOLM as the optimal inclusion level in broiler ration that significantly improved productive performance indices while reducing feed cost (Ebenebe *et al*., 2012; Gadziravi *et al*., 2012; Madrazo *et al*., 2012).

Research has shown that expensive conventional protein concentrates like soybean meal, sunflower seed cake and fishmeal can be partially replaced by MOLM in livestock and poultry feed without any adverse effect on zootechnical performance data (Ustundag and Ozdogan, 2016). Influence of supplemental inclusion of MOLM on growth indices of animals (rabbits, mice and goats) and fish has been established (Moyo *et al*., 2012; Paul *et al*., 2013). Furthermore, available information also revealed that processed moringa seed and leaf meal can successfully substitute soybean meal in poultry rations (Melesse *et al*., 2011). Kakengi *et al.* (2007) in their feeding trial observed an increase in feed and dry matter intake in chickens fed dietary MOLM, suggesting that test diet is palatable and preferred by chickens. In this regard, Onunkwo and George (2015) observed that incorporation of 10% MOLM in broiler diets had comparable performance with the group fed control diet (0% MOLM). This differs with Iheukwumere *et al.* (2007) and Onu and Aniebo (2011) who reported that inclusion of 10–15%MOLM and 2.5–7.5% MOLM improved feed intake (FI), average daily gain (ADG) and feed conversion ratio (FCR), respectively. Onu and Aniebo (2011) observed that broilers fed MOLM (7.5%) were gaining weight at no extra feed cost; however, birds on 5 and 7.5% MOLM had the best ADG and FCR. This is in disagreement with Gadzirayi and Mupangwa (2014), who reported reduced ADG and better FCR when MOLM was supplemented beyond 5% in broiler diet. Feeding broilers MOLM at 0.5% improve daily weight, feed intake and FCR, whereas birds on 1.0, 1.5 and 2.0% perform poorer in terms of FI, FCR and ADG (Divya *et al*., 2014). The administration of 120 ml of aqueous MOLE to broilers positively influenced growth indices (Alabi *et al*., 2017).

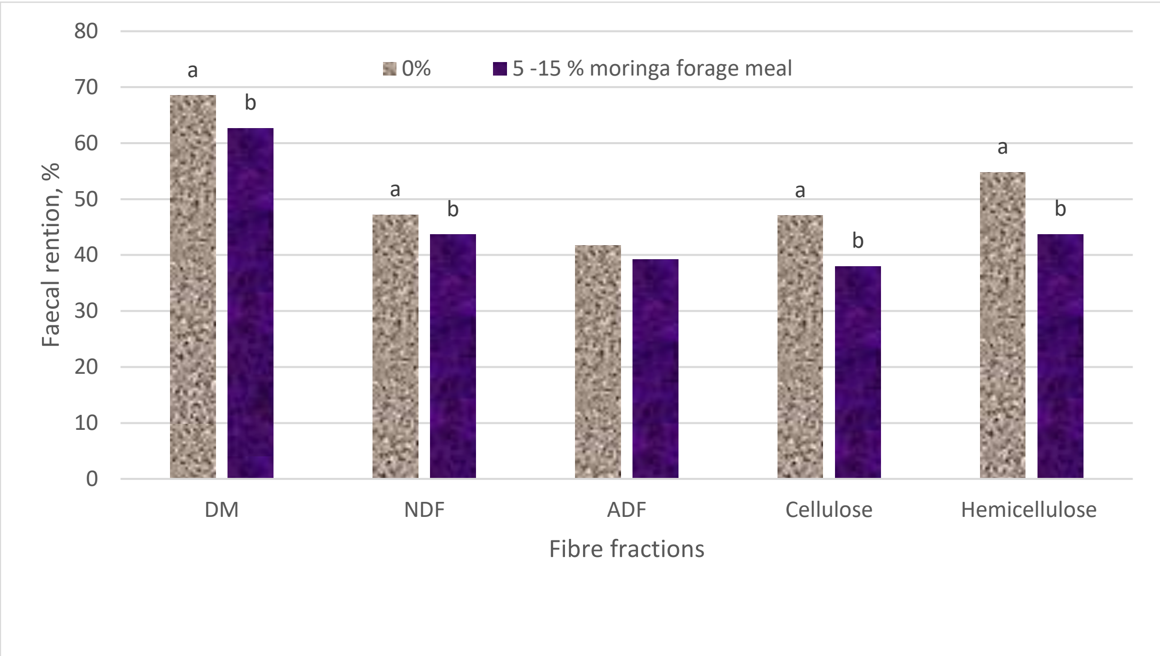

Fig. 2.2. Apparent faecal retention of the fibre fraction in broilers fed dietary MOFM Adapted from Bustamante (2014).

[a, b] Bars with the same letters are significant (p<0.05).

In studies of Moringa supplementation in animals other than broiler chickens, Gadzirayi *et al*. (2012) witnessed that indigenous chickens fed 5% MOLM as a replacement for soybean meal reduced feed intake and improve growth performance when compared with chickens that received soybean meal as the only protein source. Magouz *et al*. (2016) observed that feeding MOLM at 6, 12, 18 and 24% to Nile tilapia had no improvement effect on feed utilisation efficiency but increased the crude protein content of the whole Nile tilapia body. In a similar study in layers, Kakengi *et al.* (2007) observed increased egg production and egg mass in groups fed 20% MOLM compared with the groups on 0, 10 and 15% MOLM. However, birds on 15% and 20% MOLM had significantly higher feed intake and dry matter intake when compared with those on 0% and 10% MOLM. There was improved egg weight and FCR (kg feed/kg eggs) in birds fed 10% MOLM. Studies conducted in Cuba were in agreement with the results of others who reported that up to 10% MOLM can be added in layer diet without adverse impact on egg production and quality (Pérez, 2013; Tapia, 2013;Valdivié *et al*., 2013[a]). In a bid to save feed cost, Valdivié *et al.* (2012) have demonstrated the possibility of adding 20% MOFM in rations of one million layers aged 5–6.5 months without adverse consequence on egg production parameters. Most importantly, it was observed that the cost of feeding the one million layers during the 6 weeks' study was reduced by USD 118,334.00. However, birds on 30% MOLM did not exceed 75% egg production while those on 40% MOLM did not exceed 60% during the peak of production (Valdivié *et al*., 2013[a], 2013[b]). Interestingly, feeding MOLM at 30% and 40% favours egg yolk pigmentation (Valdivié *et al*., 2013[a], 2013[b]). Incorporation of MOLM up to 100 g/kg dry matter in goat ration did not elicit any deleterious consequence effect on performance (Yusuf *et al*., 2018). Improved ADG has been observed in rats fed fresh leaves of *M. oleifera* daily for 21 days (Osman *et al.,* 2012).

Scientific information has showcased that supplemental addition of MOLM in layer rations increased egg production and quality (Gakuya *et al*., 2014; Lu *et al*., 2016). The incorporation of MOLM at 2.5% and 5% in layer diet improved egg production and egg quality relative to those on 0% MOLM (Ebenebe *et al*., 2013). In a similar manner, Lu *et al*. (2016) observed that 5% MOLM enhanced egg yolk colour with comparable egg production when compared to layers on 0% MOLM diet. In contrast, the addition of whole seed meal of *M. oleifera* at 1, 3 and 5% improved egg yolk colour, but severely reduced feed consumption, laying ability and egg mass, thus showing that whole moringa seed meal has a negative effect on egg production and quality (Mabusela *et al*., 2018). The improved egg yolk colour in layers fed moringa– based diets may be credited to the high carotenoid content in MOLM (Saini *et al*., 2013, 2014). However, there seems to be a negative relationship between increased egg yolk pigmentation and laying performance. Therefore, more research effort should be directed at ascertaining the relationship between the increase in egg yolk colouration and egg production rate in laying birds fed MOLM–based diets. The inclusion of MOLM beyond 15% in layer diet should be executed with caution as the majority of the studies have shown that inclusion of MOLM beyond 15% has a negative influence on egg production and quality (Kakengi *et al*., 2007; Olugbemi *et al*., 2010; Abou-Elezz *et al*., 2011).

2.26 Organ anatomy, carcass yields and product quality

The influence of fibre on the maintenance of healthy gut in chickens is well reported in literature (Haoyu, 2013). However, the inclusion of fibre beyond the levels the birds can handle will lead to poor nutrient digestibility and performance Bustamante *et al*. (2013) noted a decline in the sizes of digestive tracts and internal organ in broilers receiving MOLM-based diets at 5 – 15% relative to those on the control diet (Fig. 2.3), although the observed reductions were not significant. The disparity between the control birds and those on MOLM diets may be partly explained by the physicochemical properties of the diets as well as the adaptation features of the chicken. The inclusion of 1.2% MOLM in broiler diet increased the villus height in the different parts of the small intestine, villus surface area of the duodenum and villus height, crypt depth ratio of the ileum in contrast to the broilers on control diet (Khan *et al*., 2017). Total goblet cell count in the duodenum for the treated groups was increased in comparison with the non-supplemented group (Khan *et al*., 2017). These results are in tandem with the earlier reports of others that MOLM increases the length of the villi across the three anatomical parts of the small intestine in broilers (Tesfaye *et al*., 2013; Nkukwana *et al.*, 2014a; Odedeyi *et al*., 2014). The observed increase in the intestinal length, as well as the height of the villus, could be attributed to prolonged stay-time of digesta elicited by high fibre level of the feed containing varying levels of MOLM. The increased stay-time of digesta suggests improved feed digestion and absorption of nutrients (Awad *et al*., 2009) and therefore increased weight gain.

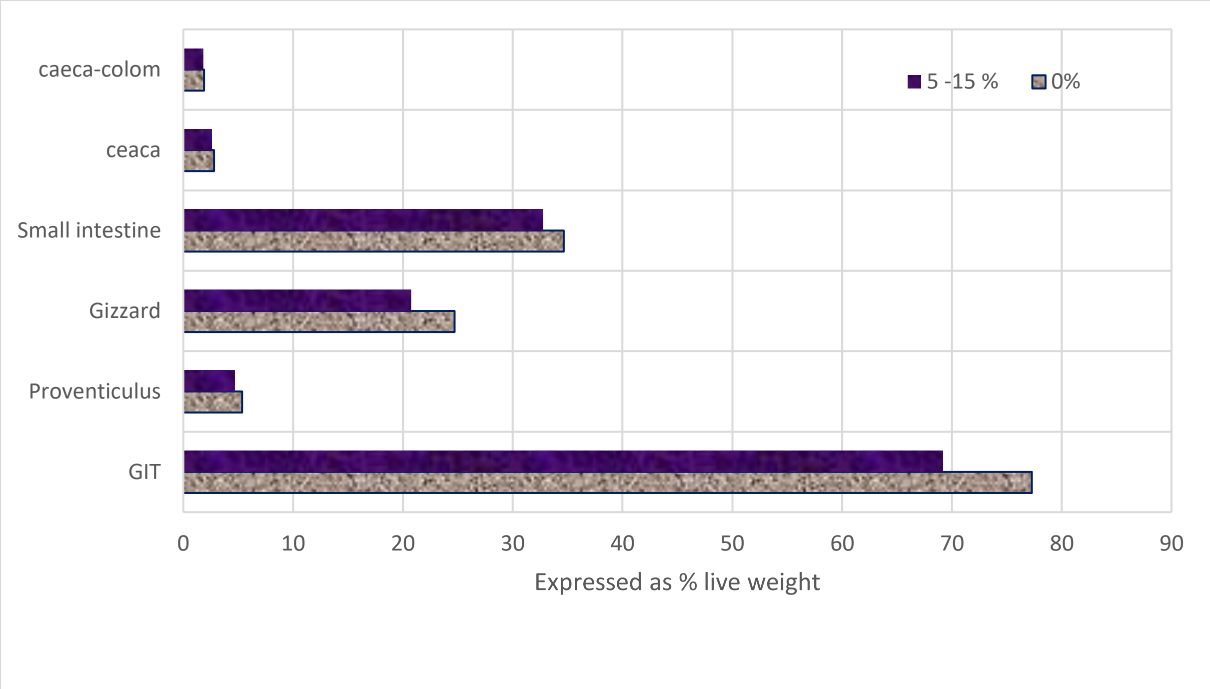

Fig. 2.3. Weights of the digestive tract and internal organs expressed as percentages of live body weight of chickens fed diets with 5 - 15% MOFM
Adapted from Bustamante *et al.* (2013)

Hassan *et al.* (2016) revealed that incorporation of MOLM at 0.1 to 0.3% in the diet of matured broilers supported organ and carcass development. These findings corroborate Nuhu (2010) and Ayssiwede *et al.* (2011), who noticed similar carcass and organ weights in growing rabbits and native chickens fed MOLM, respectively. Similar observations have been made on broilers fed MOLM at 0.1–2.5% (Nkukwana *et al*., 2014[a]). A recent feeding trial by Cui *et al*. (2018) showed that inclusion of MOLM (0–15%) in broiler diets elevated the level of the following polyunsaturated fatty acids (PUFAs) - linoleic, linolenic and arachidonic in breast muscle and reduced abdominal fat level. This is in concordance with the studies of Nkukwana *et al.* (2014[b]) who observed improved level of PUFAs in breast muscle in broilers fed MOLM–based ration. The influence of diet on PUFA content of the meat has been reported (Woods, 2009), and hence, the moderate level of PUFA in moringa leaves may be the cause for the increase in the PUFA value of breast muscle recorded in broilers fed 1–15% MOLM.

Furthermore, there is documented evidence (Mancini and Hunt, 2005) that majority of consumers' animal product buying decisions are mainly influenced by meat colour. Feeding MOLM based diets to birds influences the colour of egg yolk and broiler meat (Mukumbo *et al*., 2014; Shah *et al*., 2015). Under stressed condition, excessive production of free radicals beyond what the endogenous antioxidant enzymes can regulate leads to brownish colouration meat from its normal red colour (Falowo *et al*., 2014). Improved retention of the normal meat colour (red) in broilers fed moringa products may be ascribed to the high antioxidant activity of compounds in MOLM. Empirical studies have shown that fat and fatty acid content are vital factors that determine consumers' perception of meat quality. Consumption of meat with high fat content especially saturated FAs has been linked with increased incidence of cardiovascular diseases (Nantapo *et al*., 2015). Importantly, the inclusion of MOLM in poultry diet aids in balancing the ratio of omega 6 and omega 3 fatty acids (FA) in broiler meat to a tolerable limit for human use. Adding 5% MOLM in broiler diets improved the level of omega-3 FAwhile reducing the ratio of omega-6 to omega-3 FAs breast meat (Nkukwana *et al*., 2014[a]).

2.7 Gut health

The extract of moringa leaf has been shown to inhibit the growth of *Staphylococcus aureus* in the feed and animal intestines (Djakalia *et al*., 2011). Divya *et al.* (2014) conducted a feeding trial to ascertain the effect of MOLM at 0, 5, 10, 15 and 20 g/kg feed on gut microbiota of 420-day-old broiler chicks reared for 42 days. The authors observed that microbial load and coliform population were lower in group that received MOLM diets when compared with the control and the group with 20 mg/kg bacitracin methyldisalicylate. This reduction may be attributed to the presence of phytochemicals and essential oils contained in moringa leaves (Chuang *et al.,* 2007). The administration of aqueous *M. oleifera* leaf extract has been shown to significantly reduce the populations of *Escherichia coli* and *Clostridium* species in the guts of non-ruminants (El-Kholy *et al.,* 2018). In species other than poultry, Abalaka *et al.* (2012) observed that *E. coli* were the most susceptible microbes to the antibacterial activity of *M. oleifera* in rabbits. Similarly, Baurhoo *et al.* (2009) reported improved populations of beneficial bacteria, lactobacilli and bifidobacteria and reductions in the population of destructive microbes in the caeca of chicks that were fed fibre-rich diets. Chongwe (2011) observed a reduction in the microbial population in intestine of indigenous chickens fed MOLM at 20 and 30%.

2.8 Blood characteristics

2.81 Haematology

Blood is an index of nutritional quality in farm animals (Makanjuola *et al.,* 2014). Information on the health status of broilers fed MOLM–based diet revealed improved blood quality (Stevens *et al*., 2015) as shown in Fig. 2.4. In a 21-day feeding study, Osman *et al*. (2012) revealed that *Moringa oleifera* has a positive influence on blood parameters of the small animal model (an animal with a disease either the same as or like a disease in humans). Addition of MOLM at 5, 10 and 15% in broiler diets improved haemoglobin (Hb) concentration, red blood cell (RBC) count, packed cell volume (PCV), mean cell volume (MCV) and mean cell haemoglobin concentration (Alnidawi *et al*., 2016). Similar results have been recorded in growing rabbits fed MOLM at the same inclusion rates (Ahemen *et al*., 2013). Increased white blood cell (WBC) and lymphocytes were found in animals fed 10 and 15% MOLM (Ahemen *et al*., 2013). This is in harmony with a more current finding in broilers fed MOLM at 5, 10, 15 and 20% (Alnidawi *et al.,* 2016). Positive influence of dietary MOLM on PCV and RBC of broilers has been reported (Onu and Aniebo, 2011). This is a pointer to the fact that MOLM is rich in proteins and may be served as a feed raw material in broiler ration without causing any harmful effect on blood indices. Furthermore, a similar study in goats showed that addition of 50 g/kg DM of MOLM improves Hb concentration, PCV, RBC and WBC (Yusuf *et al*., 2018). Feeding assessment carried out by Tijani *et al*. (2015) revealed that inclusion of 5% MOLM in broiler diet yielded similar PCV value as the control birds, but birds on 10– 20% MOLM had significantly reduced PCV. Tijani *et al.* (2015) observed that broilers on 5–15% MOLM had improved ($p < 0.05$) WBC level compared with birds on control treatment (Fig. 2.5). The addition of 3 and 5%MOLM in broiler diet for 7 weeks improved PCV, WBC, Hb and RBC count (Safa and Ibrahim, 2014), thus supporting the earlier results of Onu and Aniebo (2011) that 5% may be the optimal inclusion rate of MOLM that optimises production indices in broilers.

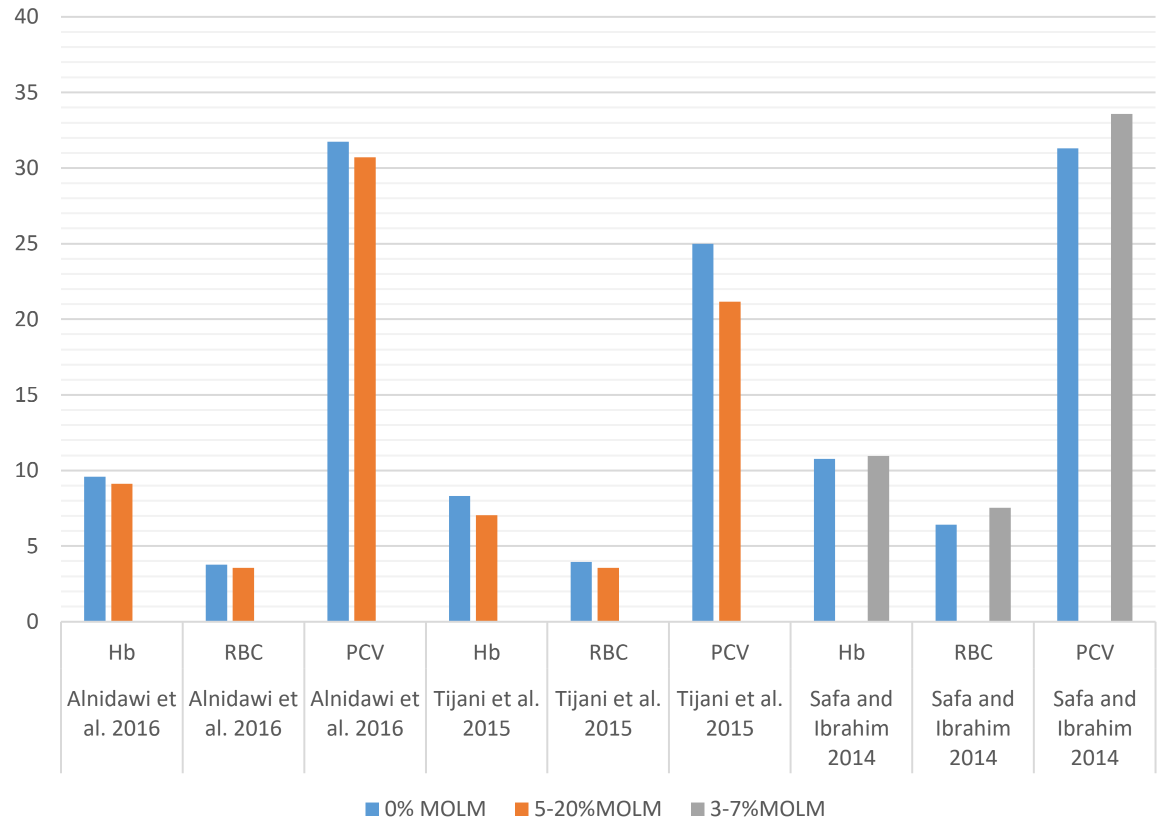

Fig. 2.4. Hb, RBC and PCV values of broiler chickens on different inclusion levels of dietary MOLM.

Adapted from Safa and Ibrahim (2014), Tijani *et al.* (2015) and Alnidawi *et al.* (2016).

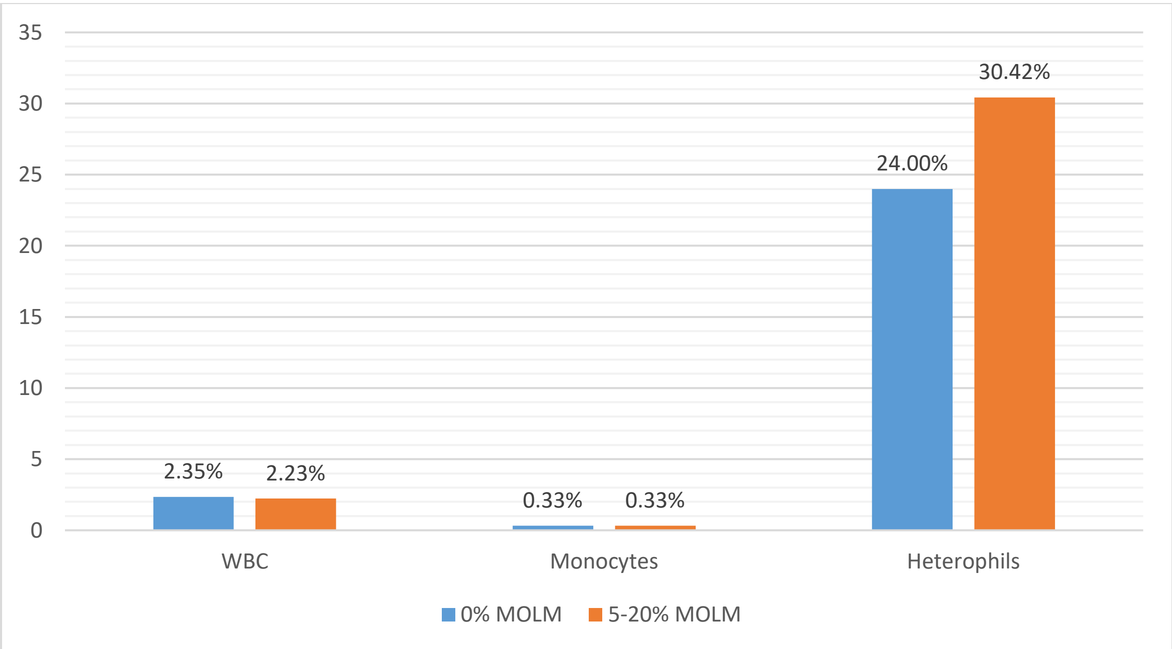

Fig. 2.5. The WBC indices of broilers fed MOLM based diet at 0% and 5 - 20%

Adapted from Tijani *et al*. (2015)

2.82 Serum biochemistry

MOLM supplementation (0, 0.5, 1.0, 1.5 and 2.0%) had a mild reduction effect on total serum protein, triglyceride, cholesterol, albumin and uric acid in broiler chickens (Divya *et al*., 2014). In the case of liver cell damage, aspartate transaminase (AST) and alanine transaminase (ALT) are leaked out of the liver to the serum (Enemor *et al*., 2005). Also, the addition of MOLM at 0.5, 1.0, 1.5 and 2.0% reduces the concentrations of liver enzymes (AST and ALT) in the sera, hence indicating the absence of liver damage as a result of the feeding of test diets. Creatinine, a waste product of protein catabolism in the body, was noticed to decline with increasing levels of MOLM in the diet, thus suggesting that birds on MOLM-based diets may not be in a state of negative energy balance. In variance to the earlier postulation of Divya *et al.* (2014) that MOLM reduces serum protein level in broiler chickens, Safa and Ibrahim (2014) and Yusuf *et al*. (2018) observed an increased total serum protein in WAD goats fed MOLM at 3–5% and 50–100 g/kg DM feed, respectively (Fig. 2.6). The disparity may be ascribed to the quantity of MOLM added in the diet and species difference as goats are ruminants and have the ability to effectively utilise fibrous leaf meals. Improved total serum protein observed in broilers fed MOLM–based diet at 2.5% and 5.0% has been reported (Onu and Aniebo, 2011). Alnidawi *et al*. (2016) observed the hypolipidaemic and antihypercholesterolemic property of MOLM (Fig. 2.7). This differs with the previous reports of Sangkitikomol *et al.* (2014) that MOLM supplementation inhibits lipid biosynthesis. Blood biochemical indicators (protein, uric acid, AST and ALT) have been reported to decline in birds fed 20% MOLM (Tijani *et al*., 2015).

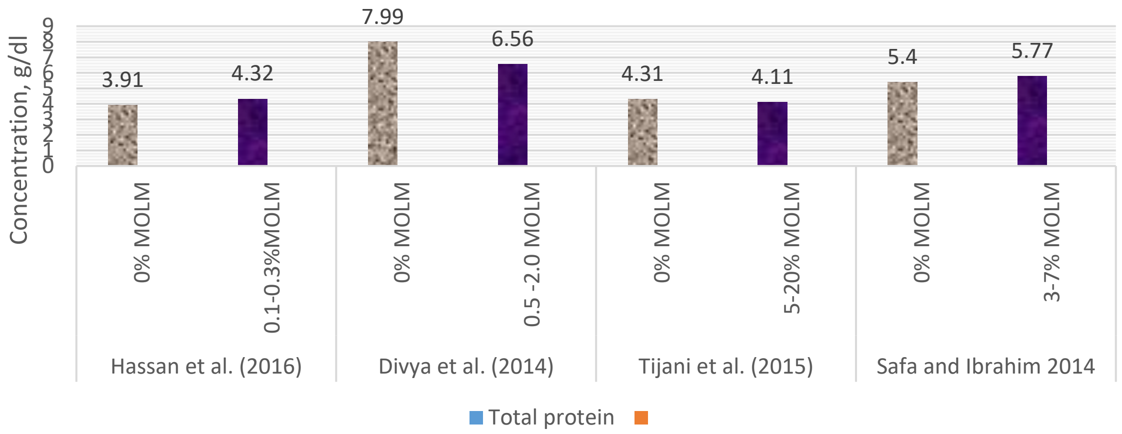

Fig. 2.6. The total serum protein values of broiler chickens on graded levels of dietary MOLM Adapted from Divya *et al.* (2014), Safa and Ibrahim (2014), Tijani *et al.* (2015) and Hassan *et al.* (2016).

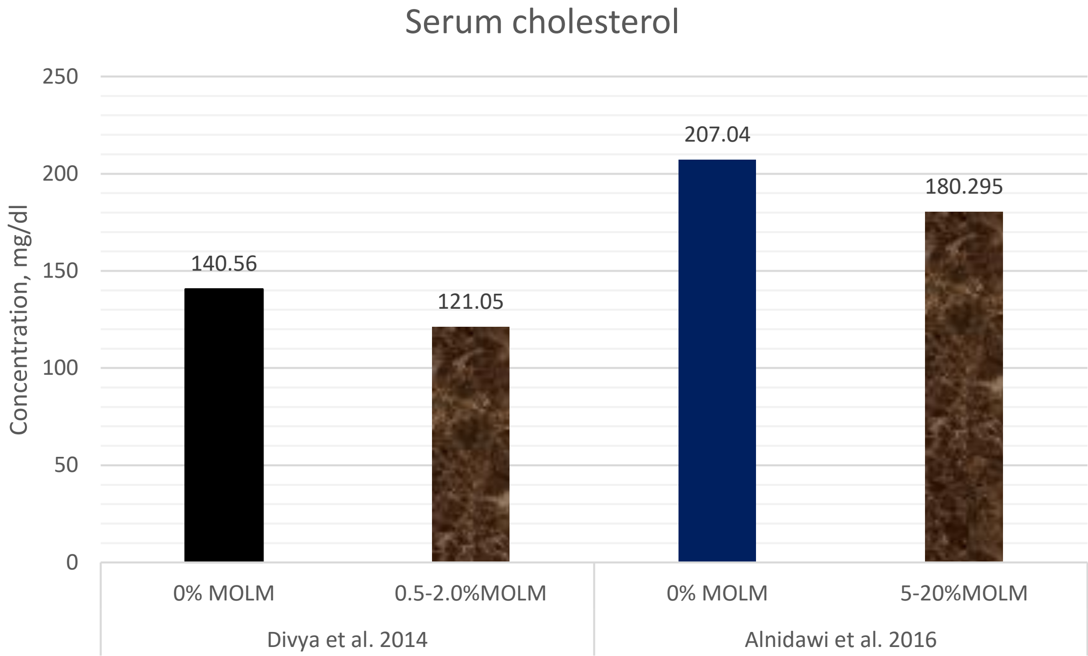

Fig. 2.7. The total serum cholesterol level of broilers fed graded levels of dietary MOLM Adapted from Divya *et al.* (2014) and Alnidawi *et al.* (2016).

2.9 Conclusion

In conclusion, *M. oleifera* is an excellent source of important bioactive ingredients and nutrients that may be exploited for broiler production. However, further studies are needed to identify and isolate the actual bioactive compounds that are accountable for the growth promoting ability, as well as the antihypercholesterolemic, antihyperlipidaemic and antioxidant activity of moringa leaf meal which may lead to the development of new therapeutic compounds. Another focal area is to evaluate the commercial use of M*. oleifera* leaf meal in pigmentation of egg yolk and broiler meat. However, there tends to be a negative correlation between improved egg yolk colouration and laying performance in laying birds fed high levels of moringa product–based diets; hence, more research is needed in this direction. The ability of dietary MOLM to improve body weight gain, immune system, gut health and reduce blood cholesterol levels in broilers as documented may have been actualized via the following mechanisms of action: (i) stimulate gut health and defence system via selective exclusion antagonism; (ii) increase the villi length and width, hence leading to a better absorptive ability of the different segments of the small intestine; (iii) direct nutritional effects; (iv) activation of gut defence system; and (v) stimulation the production and release of glutathione concentration which in turn reduces cellular damage by scavenging for free radicals. The application of transcriptomics technologies in the explanation of the actual mechanisms behind the growth-promoting effect and health benefits of MOLM in broiler production is strongly advocated because for now there is no such information in the literature

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CHAPTER 3

MINERAL COMPOSITION OF MORINGA OLEIFERA LEAF MEAL (MOLM) AND THE RESPONSES OF ROSS 308 BROILERS TO MOLM SUPPLEMENTATION

3.1 Introduction

The development of the broiler industry as a means of bridging the food and nutrition insecurity gap in most countries has been attracting great attention (Dieye *et al.*, 2010). Unfortunately, the growth of the broiler sector in most developing countries are constrained by the spiralling feed cost due to rising prices of feed ingredients particularly protein supplements (Abbas, 2013). This spiralling feed cost is having negative impacts on the feed supply chain as well as the quality of the compounded rations. This rising price of feeds has been partly attributed to the competition between humans and animals for protein and energy concentrates. Therefore, it has become imperative that the potential of alternative protein feedstuffs that are not in direct competition with humans be fully harnessed in broiler nutrition (Ogbuewu *et al*., 2015; Ogbuewu *et al*., 2019). One of the best-suited alternative plant protein feed raw material for broiler feeding in the developing countries is the leaf of Moringa plant.

Moringa oleifera belongs to the family Moringaceae and thrives well during severe drought and heat. *Moringa oleifera* is one of commonest specie among the 14 species in the family and grows to a height of 7 – 12 m. It is native to India, but thrive well in the tropics (Nsofor *et al.,* 2012). The leaves of moringa plant are high in essential amino acids, protein, minerals, vitamin A and fibre (Ustundag and Ozdogan, 2015) and could be incorporated in broiler feed. In addition, *M. oleifera* leaf meal contains 2.4% ether extract, 25.4% crude protein, 17.4% crude fibre and 5.9% ash (Alnidawi *et al*., 2016) as against 18.8% ether extract, 42.8% crude protein, 20% crude fibre and 5.5% ash in soybean meal (Banaszkiewicz, 2000). *Moringa oleifera* leaf therefore is lower in protein than soybean but much higher in ash (minerals) value thus supporting the earlier reports of Ustundag and Ozdogan (2015) that *M. oleifera* leaf can partially substitute soybean and fishmeal in broiler rations without negative impacts of production data while lessening the prize of feed (Ebenebe *et al*., 2012). In addition, research has established that *M. oleifera* leaves are abundant in water and fat-soluble vitamins as well as lutein and β-carotene (Igwilo *et al*., 2014; Saini *et al.*, 2014). Evidence also exists that *M. oleifera* leaf is a storehouse for a variety of essential bioactive elements such as saponins, flavonoids, and phenols among others (Saini *et al*., 2016).

Feeding trial conducted by Kakengi *et al.* (2007) in chickens fed *M. oleifera* leaf meal revealed enhanced feed and dry matter intake hence showing the palatability of the *M. oleifera* leaf meal based diets to the chickens. Other authors (Onu and Aniebo, 2011; Onunkwo and George, 2015) fed broilers diet containing 10% MOLM and noticed a boost in feed utilization and weight gain in comparison with birds fed 0% MOLM. In an experiment conducted in native chickens, Gadzirayi *et al.* (2012) observed reduced feed consumption and enhanced growth rate in birds that received MOLM at 5% as a substitute for soybean meal (SBM) when compared with the group fed SBM alone as the main protein source. Increased egg production and egg mass in laying hens fed 20% MOLM has been documented (Kakengi *et al*., 2007). Also, improved growth rate and blood constituents in goats and fish fed MOLM have been reported (Paul *et al*., 2013; Jiwuba *et al*., 2017).

Several publications have been documented on the use of Moringa leaves as a feed resource for livestock and poultry (Gadzirayi *et al*., 2012; Paul *et al*., 2013; Onunkwo and George, 2015; Jiwuba *et al*., 2017). However, there is comprehensive data on the mineral assay of moringa leaves and such knowledge will allow farmers to optimize the nutritional value of this plant in animal production. Hence, it has become important to investigate the mineral constituents of leaf meal of *Moringa oleifera* plant and their influence on the production characteristics of meat typed chickens. The objectives of this feeding trial therefore, was to determine the mineral content of *M. oleifera* leaf meal and the effect of its supplementation on production physiology of Ross 308 broilers. The *M. oleifera* leaf meal supplementation level that supported optimum performance in Ross 308 broilers were also reported.

3.2 Materials and Methods

3.21 Study site and preparation of *Moringa oleifera* leaf meal

The study was performed in a private broiler farm located at Plot 519 Zuurbekom, Gauteng Province, South Africa in the months of December 2017 to January 2018. Gauteng Province is located at the Highveld of South Africa and lies between latitude 26.2708^0 S and longitude 28.1123^0 E. The mean annual rainfall of the study location is 790 mm while the mean ambient temperature ranged between 15 to 26^{o}C in summer and 4 to 20^{o}C in winter. Fresh and tender leaves of *M. oleifera* were harvested in Grootfontein, a village near Polokwane in Limpopo Province of South Africa and air-dried for 3-5 days to a moisture level of about 10%. Thereafter, the dried leaves were milled and stored in unused feedbags for determination of mineral content and diet formulation.

3.22 Mineral analysis

The mineral values were determined in triplicates in the Department of Animal Science Laboratory, North-West University-Mafikeng Campus, South Africa as described by AOAC (2000). The mineral constituents determined were calcium (Ca), phosphorus (P), potassium (K), magnesium (K), sodium (Na), sulphur (S), iron (Fe), chromium (Cr) and manganese (Mn).

3.23 Experimental procedures

Day-old Ross 308 broiler chicks (n = 500) were allotted to five treatments in completely randomized design with five replications and each replicate having 20 chicks. Birds received commercial mash diets (Table 3.1) supplemented with MOLM at 0, 25, 50, 75 and 100 g/kg feed, respectively and tagged T1, T2, T3, T4 and T5. Birds were procured from the National Chicks' Hatcheries located in the Boschkop area of East of Pretoria, Gauteng Province, South Africa. The floor of each pen was swept, scrubbed with a strong brush, thereafter cleaned with water and later disinfected with Virkon® S. Pens were left open for several days after cleaning with Virkon® S in order to break the breeding cycle of the pathogens that may have escaped the actions of the disinfectant. The floor of each of the pen was covered to a depth of 7 cm with fresh sawdust under a deep litter management system. The drinkers, feeders, and accessories were assembled and carefully positioned 7 days before the chicks arrived. In addition, pens were heated for 24 hours via an infrared light before the chicks arrived. The starter diet (21.0% crude protein) and grower diet (19.0% crude protein) were formulated according to NRC (1994) standard to meet the nutrient requirements of the experimental birds. Feed and water were offered a*d libitum* and the investigation lasted for 42 days. All the biosecurity measures conformed to the rules and guidelines of the Ethics Committee of the University of South Africa and the South African Animal Disease Act 35, of 1984.

3.24 Data collection

The initial live weights (LWs) of broiler chicks in each pen were taken with an electronic weighing scale at the beginning of the study and weekly thereafter to obtain the weekly LW and weight difference. The mean LW of birds on each pen was determined by dividing total LW by the number of birds in the pen. Feed consumption was determined by subtracting the weight of leftover feed in the feeding trough the following morning from the weight of feed offered daily to the birds. Average daily gain in each pen was computed as the quotients of the LW at the end of the study less the live weights at the start of the study and the period the investigation lasted. Feed conversion ratio (FCR) was determined as the quotient of FI and ADG. At 42 days, 15 birds were chosen from each treatment, denied of feed for 12 hours and thereafter, slaughtered to assess the carcass and organ weights.

Table 3.1**.** Nutrient content of the diets used in the experiment.

Nutrient* (%)	Starter (0-21 days)	Grower (22 - 42 days)
Crude protein	21.0	19.0
Lysine	1.30	1.15
Methionine	0.58	0.5
Moisture	12.0	12.0
Fat	2.5	2.5
Fibre	5.0	6.0
Calcium	1.2	1.2
Phosphorus	0.6	0.55

*As illustrated in feed label

The carcass, organ and cut parts were weighed and expressed as a percentage of the live weight and carcass weight respectively following the procedures of Chukwukaelo *et al*. (2018).

3.25 Data analysis

Data collected were subjected to one way- analysis of variance, and differences in means where significant F-test is ($p<0.05$), Duncan's multiple range test (Duncan, 1955) method was used to separate the means (SPSS 24.0 package). The supplementation related responses in growth and production parameters to MOLM in Ross 308 broilers were modeled using the following quadratic optimization equation:

$Y = a + b_1x + b_2x^2$,

Where

Y = growth indices (LW, FI, ADG, FCR and mortality), carcass, dressing percent, cut parts (breast, thigh and drumstick) or organ (liver, heart and gizzard) weight;

a = the Y intercept;

b = coefficient of quadratic optimization equation;

x = MOLM supplementation levels and $-b_1/2b_2$ = x value for optimum response.
The quadratic equation was fitted to the experimental data by means of the nonlinear model procedure of SPSS 24.0. The choice of the quadratic regression model is because it fitted the model and the probability level for significance at 5%.

3.3 Results

3.31 Nutrient composition of experimental diet and MOLM

The results of the nutrient content of the treatment diets are shown in Table 3.1. The crude protein levels were 21.0% for the starter mash and 19.0% for the grower mash. Table 3.2 presents the mineral composition of MOLM. The MOLM contains appreciable levels of beneficial minerals, including calcium, phosphorus, magnesium, sodium, potassium, iron, sulphur, chromium and manganese showing that MOLM is a source of mineral for livestock. The coefficient of variation (CV) values across sample measurements for the minerals were higher and ranged from 61.35 – 81.70%.

3.32 Growth performance of starter broilers

Table 3.3 presents the growth indices of starter Ross 308 broilers fed diets supplemented with varying levels of MOLM. Broilers fed diet T1 recorded the highest FLW of 833 g, which was significantly different from birds on diets supplemented with MOLM at 25, 50, 75 and 100

Table 3.2. Macro and micro mineral composition of MOLM (mg/kg)

Parameters	Mean ± SD	CV (%)
Calcium	2687.41 ± 188.68	70.20
Phosphorus	120.73 ± 8.68	71.85
Magnesium	250.00 ± 17.88	71.51
Sodium	968.02 ± 6.03	62.32
Potassium	734.59 ± 45.10	61.35
Iron	316.98 ± 31.68	73.73
Sulphur	1144.09 ± 87.52	76.44
Chromium	3.21 ± 0.26	81.7
Manganese	12.52 ± 0.14	67.18

SD – Standard deviation; CV – Coefficient of variations

Table 3. 3. Growth indices of starter broilers (1-3 weeks) fed MOLM supplemented diets

MOLM Level	ILW (g)	FLW (g)	DFI (g)	ADG (g)	FCR	Mortality (%)
0 g/kg feed	40.73	833.87^{a}	34.59	37.77^{a}	2.29	3.00^{a}
25 g/kg feed	39.64	771.56^{b}	32.46	34.85^{b}	2.40	0.00^{c}
50 g/kg feed	40.10	770.49^{b}	32.80	34.78^{b}	2.40	0.00^{c}
75 g/kg feed	40.03	748.72^{b}	32.86	33.75^{b}	2.43	1.00^{b}
100 g/kg feed	39.39	728.30^{b}	31.19	32.81^{b}	2.41	0.00^{c}
Mean	39.98	770.59	32.78	34.79	2.39	0.80
SD	0.51	39.60	1.22	1.86	0.06	1.30
CV (%)	1.28	5.13	3.70	5.35	3.96	-
SEM	0.23	17.71	0.54	0.83	0.03	0.58
p-value	0.06	0.01	0.06	0.02	0.14	0.02

Means with the same letters within column differed statistically at p<0.05, ILW – initial live weight, FLW – final live weight, DFI – daily feed intake, ADG – average daily gain, FCR – feed conversion ratio, SD - standard deviation, CV- coefficient of variation, SEM – standard error of the mean. FCR = Food consumed/Weight gain

g/kg feed. ILW and DFI had similar (p>0.05) value in the groups. ADG decreased (p<0.05) steadily with increasing supplementation levels of MOLM. Birds on diet T1 (0 g MOLM/kg feed) had better FCR when compared with birds that received diets supplemented with MOLM at 25, 50, 75 and 100 g/kg feed, however, the difference was not significant (p>0.05). Mortality rate was significantly (p<0.05) higher for birds on diet T1 when compared with birds fed the other four diets.

3.33 Growth performance of finisher broilers

The result of growth indices of finisher Ross 308 broilers fed diets supplemented with varying levels of MOLM are shown in Table 3.4. Higher FLW (p>0.05) was reported control birds fed MOLM at 0 g/kg feed than those fed diets supplemented with MOLM at 25, 50, 75 and 100 g/kg feed. Broilers fed MOLM supplemented diet at 0, 25, 50 and 100 g/kg feed had comparable (p>0.05) ILW, DFI, and ADG values. Birds fed MOLM supplemented diet at 75 g/kg feed had the best FCR, although the difference was not significant (p>0.05) compared with the groups fed the other four diets. Birds on 0 g/kg feed MOLM had the highest mortality ($p<0.05$) when compared to the birds supplemented with 25, 50, 75 and 100 g/kg feed MOLM.

3.34 Carcass and organ weight of finisher broilers

The performance in terms of the relative weights of the carcass and organs are presented in Table 3.5. Birds fed diet with MOLM at 0 g/kg feed had higher live weight ($p<0.05$) than those on diets supplemented with MOLM at 25, 50, 75 and 100 g/kg feed. Birds on diet with MOLM at 0 g/kg feed had higher non-significant ($p>0.05$) carcass weight than those on diets with MOLM at 25, 50, 75 and 100 g/kg feed, although, broilers on diets with MOLM at 75 g/kg feed returned the highest non-significant dressing percent ($p>0.05$) relative to the birds on the other four diets. Cut parts and organ weights of broilers fed MOLM supplemented diets at 25, 50, 75 and 100 g/kg feed had the same values.

3.35 Optimization functions

The results of the effect MOLM supplementation on FLW and ADG in Ross 308 broilers are presented in Table 3.6 and Table 3.7. FLW and ADG were optimized at 2538.4 – 173.97 MOLM + 21.756 MOLM^2, $r^2 = 0.8994$ and 79.334 - 5.8806 MOLM + 0.8214 MOLM^2, $r^2 = 0.8960$ respectively in starter broilers while FLW was optimized at 3173.7 - 200.36 MOLM + 21.371 MOLM^2, $r^2 = 0.9928$ in finisher broilers.

Table 3.4. Growth indices of finisher broilers (4 - 6 weeks) fed MOLM supplemented diets.

MOLM Level	ILW (g)	FLW (g)	DFI (g)	ADG (g)	FCR	Mortality (%)
0 g/kg feed	763.87	3000.40^{a}	124.57	74.76	1.54	5.00^{a}
25 g/kg feed	761.56	2848.40^{b}	122.69	69.70	1.40	0.00^{b}
50 g/kg feed	760.49	2761.20^{b}	122.16	69.65	1.63	0.00^{b}
75 g/kg feed	768.72	2729.33^{b}	123.38	69.36	1.31	2.00^{b}
100 g/kg feed	768.30	2699.26^{b}	121.90	70.17	1.41	0.00
Mean	764.59	2807.72	122.94	70.73	1.46	1.40
SD	4.60	121.30	1.07	2.27	0.13	2.19
CV (%)	5.13	4.32	0.87	3.21	8.66	156.48
SEM	5.71	55.25	0.48	1.02	0.06	0.98
p-value	0.610	0.038	0.75	0.82	1.14	0.02

Means with the same letters within column differed statistically at $p<0.05$, ILW – initial live weight, FLW – final live weight, DFI – daily feed intake, ADG – average daily gain, FCR – feed conversion ratio, SD - standard deviation, CV- coefficient of variation, SEM – standard error of the mean.

Table 3.5. Carcass and organ weight values of finisher broilers fed MOLM diets

	MOLM inclusion (g/kg feed)					Mean	SD	CV	SEM	P-value
Parameters	0	25	50	75	100					
LW (g)	3000.40[a]	2848.40[b]	2761.20[b]	2729.33[b]	2699.26[b]	2807.72	121.30	4.32	55.25	0.0384
CW (g)	2911.53	2778.93	2690.53	2659.60	2637.86	2735.69	112.01	4.09	50.09	0.1128
BW (%CW)	27.26	25.54	30.02	25.27	27.42	27.10	1.90	7.00	0.85	0.3960
DW (%CW)	16.81	17.11	18.02	18.65	18.64	17.85	0.85	4.78	0.38	0.5208
TW (%CW)	21.23	22.83	22.90	23.73	23.66	22.87	1.01	4.49	0.45	0.6315
HW (%LW)	3.01	1.94	2.28	2.08	2.04	2.27	0.43	19.01	0.19	0.0828
LW (%LW)	9.26	9.76	10.13	10.46	10.54	10.03	0.53	5.27	0.24	0.9785
GW (%LW)	7.87[b]	9.19[a]	8.56[ab]	8.56[ab]	10.21[a]	8.88	0.88	9.89	0.39	0.2358
DP (%)	97.04	97.56	97.42	97.45	97.71	97.44	0.25	0.25	0.11	0.0781

Means in the same row sharing the same superscript are significant ($p<0.05$). LW – live weight, CW – carcass weight, BW- breast weight, DW – drumstick weight, TW – thigh weight, HW – heart weight, LW – liver weight, GW – gizzard weight, DP – dressing percent, SD - standard deviation, CV- coefficient of variation, SEM – standard error of the mean.

Table 3.6**.** Quadratic analysis of the effect of MOLM on FLW and ADG in starter broilers

Variables	Formular	r^2	x-value	y-value	P-value
FLW	$Y = 2538.4 - 173.97x + 21.756x^2$	0.8994	39.98	2190.63	0.0100
ADG	$Y = 79.334 - 5.8806x + 0.8214x^2$	0.8960	35.80	68.81	0.0200

Table 3.7. Quadratic analysis of the effect of MOLM on FLW in finisher broilers

Variable	Formular	r^2	x-value	y-value	P-value
FLW	$Y = 3173.7 - 200.36x + 21.371x^2$	0.9928	46.88	2704.09	0.0384

3.4 Discussion

The objective of this feeding investigation was to determine the responses of Ross 308 broilers to commercial diets (starter and grower) mash supplemented with varying levels of MOLM. The starter diet contained crude protein level of 21.0% while grower contained crude protein level of 19.0% with similar levels of other essential nutrients that satisfied the bird's nutrient demands as endorsed by NRC (1994) for poultry.

Minerals are vital in poultry nutrition because they serve as cofactors for several biological processes. Calcium, an important mineral for bone growth, muscle, and neurological functions is high in MOLM with the order of macro mineral level being calcium (2687.41 mg/kg) > sodium (968.02 mg/kg) > potassium (734.59 mg/kg) > magnesium (250.00 mg/kg) > phosphorus (120.73 mg/kg) as determined in the current study. These observations are in harmony with the value recorded by Sena *et al.* (1998). Furthermore, the calcium, potassium, magnesium and phosphorus values determined herein were lower in general than the corresponding values of 16046.7, 17450, 2833.8 and 4827.4 mg/kg published by Olson *et al*. (2016) in Mexico. Freiberger *et al.* (1998) and Yaméogo *et al.* (2011) have noticed a higher calcium value (14,400 to 35,126 mg/kg) than the value determined in this experiment. It is therefore not clear what is responsible for this wide difference. Noteworthy too was the fact that the sodium content (117.4 mg/kg) reported by Olson *et al*. (2016) was about 99% units lower to what was determined in this experiment. These variations may be associated with such factors as soil composition, agro climatic condition, plant age and stage of maturity of the leaves, digestion protocols and analyzing techniques used as observed by Melesse *et al*. (2012) and Mbah *et al.* (2012). Phosphorus is another important macro mineral needed for rapid bone growth in meat-typed chickens. Such rapid bone growth requires adequate calcium and phosphorus supply (Williams *et al.*, 2000).

Inadequate supply of both or one of the minerals as a result of the deficiency or excess of one of them interferes with homeostasis of the second one (Kebreab and Vitti, 2005), resulting in reduced growth rate and bone mineralization (Hurwitz *et al*., 1995). The analysed leaf meal contained small amounts of phosphorus; however, this was not a problem since the leaves were used as protein supplement for broilers. The study plant also contains appreciable quantities of iron and sulphur. This high iron content could explain the improved blood values in chickens fed MOLM based diets (Ustundag and Ozdogan, 2015; Alnidawi *et al*., 2016) since they are needed for haemoglobin formation. Noteworthy is that MOLM has high potential as a good source of dietary minerals (calcium, potassium, sodium, iron, and sulphur) in animal nutrition. High sulphur content (1144.09 mg/kg) recorded in the current study for MOLM was in agreement with Lyon *et al.* (2017) who reported that *Moringa oleifera* tree has an exceptional power to take up and store mineral sulphur in the leaves, even when grown on soils low in sulphur. The health and medical benefits of this finding is that MOLM may be used to enhance productivity in animals fed low sulphur diets. The coefficient of variation of mean mineral values as observed in the current study was high, thus suggesting that the means could not serve as reference mineral value for MOLM in the study location.

The results reveal that the final live weight of starter broilers on the control birds was significantly higher than the groups on diets supplemented with MOLM at 25, 50, 75 and 100 g/kg feed, but lower than the reference live weight of 929 g recorded for 21-day-old unsexed Ross 308 broilers (www.aviagen.com). Factors such as enhanced nutrition and improved housing conditions may have explained the observed disparity in starter phase. The present result showed that MOLM supplementation up to the rate of 100 g/kg feed showed no adverse influence on feed consumption. This compared favourably with the result of Divya *et al*. (2014) who also noticed non-significantly reduced feed intakes in broilers fed *Moringa oleifera* leaf meal based rations. This finding indicate that the level of MOLM supplementations used in the current study are acceptable to the birds. However, the reason for the acceptability is not known. Although, one possible reason is anti-nutritional factors as similarly observed by Makkar and Becker (1996). The non-significant reduction in average daily gain as observed for birds fed MOLM supplemented diets in the current feeding investigation was similar to value recorded in broilers by Divya *et al.* (2014). There was no significant MOLM effects on feed to gain ratio in the present feeding investigation. The result of the percentage mortality revealed that MOLM supplementation significantly influenced mortality rate with birds on the control diet experiencing the highest rate. Mortality was significantly low in treatment groups relative to the control group and this observation may be because of high levels of phytochemical compounds in moringa leaves, which have been reported to have medicinal and pharmacological property such as antiprotozoal, antibacterial and antifungal effect as well as immune stimulating actions in animals (Sharma and Paliwal, 2013). Furtherance to this, the coefficient of variation of mean growth performance values were small, indicating that the reported means could be used as reference value for broilers fed diets with varying supplementation levels of MOLM in the study location.

The non-significant carcass weight and dressing percent at 25, 50, 75 and 100 g/kg feed supplementation rates can be attributed to high quality of the MOLM supplemented diets and enhanced nutrient utilisation by the broilers (Hassan *et al*., 2016). The result of the weights of breast, drumstick and thigh suggested that MOLM supplementation positively influenced the development of the cut parts but did not maintain progressive pattern, which is in agreement with the earlier findings published by Hassan *et al.* (2016). This observation has shown the suitability of MOLM supplemented diets for enhanced cut parts development and production in broilers and therefore may be recommended for broiler production. The slight enlargement in the size of the liver of broilers fed MOLM supplemented diets at 25 - 100 g/kg feed could be linked to enhanced activity of this organ in a bid to detoxify the antinutritional factors that may be present in the experimental diet (Igwilo *et al*., 2007). The weight of gizzard at 25 and 100 g/kg feed was significantly increased and this corroborates works of Ayssiwede *et al.* (2011), who recorded higher gizzard weights in indigenous chickens fed MOLM based diets. In addition, the coefficient of variation of mean carcass and organ parts were narrow, suggesting that the means could serve as standard value for Ross 308 broilers fed MOLM supplemented diets in the study region.

The results of the quadratic function showed that no single MOLM supplementation level optimized FLW and ADG in the current feeding experiment. The quadratic function showed that all the significant parameters had very high (89.6 – 99.3%) coefficients of determination (r^2). Handful of studies has used a quadratic model to ascertain the optimal levels of feed that supported optimum performance variables in chickens (Okoro *et al.,* 2016 a, b). However, information on the use of quadratic analysis to determining the MOLM supplementation level that supported optimum production parameters in Ross 308 broilers is lacking in the literature. The results of the quadratic function in the current study corroborate the results of Okoro *et al.* (2016a, b) who observed that no single feed inclusion level optimized all production parameters in chickens. The results of this feeding experiment revealed that for starter broilers, FLW and ADG was statistically optimized at 39.98 and 35.80 g/kg feed respectively. However, the value of 39.98 g/kg feed MOLM that optimized final live weight at the starter phase was 6.9 g/kg lower than the value of 46.88 g/kg that optimized the final live weight at the finisher phase. The observed difference is expected since the starter diet was higher in protein (21.0%) than the finisher diet (19.0%), thus the birds on the starter phase will tend to take less diet to meet their daily protein requirements when supplemented with MOLM which are reported to contains 25.4 – 42.8% crude protein by Banaszkiewicz (2000) and Alnidawi *et al.* (2016). The high r^2 value recorded for the FLW and ADG revealed the high strength of relationship of the two variables using the quadratic analysis. These observations have practical application when supplementing diets with MOLM for FLW and ADG in Ross 308 broilers as to reduce the wastage of feed supplements. The significant optimization influence on FLW and ADG n implied that their values could be predicted at a given quantity of MOLM supplemented in the broiler diet.

3.5 Conclusion

The information provided in the current investigation further support the potential of *Moringa oleifera* leaves as an alternative non-conventional protein feed resource in broiler chicken diets. Importantly, this is useful for smallholder poultry farmers who are compelled by the high prices of conventional protein feed resource such as soybean meal to rely on such alternative protein source for enhancing the productivity of their animals. In addition, *Moringa oleifera* leaves are good sources of minerals and is suitable for cut parts development and production of enhanced cut parts in the broiler chickens. It is therefore, recommended that supplementation rate of up to 100 g/kg feed MOLM may be well accepted by the Ross 308 broiler chickens.

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CHAPTER 4

MODERATE LEVELS OF DIETARY *MORINGA OLEIFERA* LEAF MEAL SUPPLEMENTATION IMPROVES BLOOD VALUES IN BROILER CHICKENS.

4.1 Introduction

The quest to improve poultry health and performance as well as the production of healthy animal products and maintenance of better environment has led to the adoption of environment-friendly practices by the poultry industry. Moringa leaves has been historically proven and the results revealed that it has been used as a medicinal plant. One of such practices is the inclusion of nutritive and non-nutritive feed additives to the feed during formulation to enhance the physicochemical properties of the diet for boosting poultry health and performance (NRC, 2001). Some of the feed additives used in livestock and poultry farming to stimulate performance are prebiotics, probiotics, organic acids and phytogenics. Research has shown that phytogenics, one of such feed additives improve fish and poultry health and production indices by improving gut health and energy metabolism, stopping the growth of pathogens and stimulating the release of essential nutrients from the feed (Lückstädt, 2008). The addition of phytogenics (e.g. moringa) in poultry feed during ration formulation to boost health and performance in place of antibiotic growth promoter have been strongly advocated by the European Union.

Moringa (*M. oleifera*) is a drought resistant plant widely cultivated in the tropics and subtropics. Moringa root is moderate in oil (6.33%) and ash (6.93%), but low in crude protein (5.02%) (Igwilo *et al*., 2014). The leaves are richly endowed with potassium and sodium but low in calcium (Tijani *et al*., 2015; Ustundag and Ozdogan, 2016). Likewise, the leaves, the seeds are also rich in potassium and sodium content (Tijani *et al*., 2015; Ustundag and Ozdogan, 2016). The calcium, sodium and potassium content of moringa leaves are higher than those in pumpkin and amaranths (Tijani *et al*., 2015). It has been observed that moringa leaf and seed has all the 13 essential amino acids (EAAs) needed for body maintenance and growth in poultry (Fuglie, 2001; Igwilo *et al*., 2010), however, the leaf is high in isoleucine and glutamate (Chelliah *et al.*, 2017). Moringa leaf is a storehouse for retinol, tocopherol, vitamin C and carotenoids (Igwilo *et al*., 2010).

Authors (Ogbe and Affiku, 2011; Alabi *et al.,* 2017) have disclosed that moringa contains several essential active ingredients such as flavonoids and phenols which may be useful in broiler production. Feeding moringa-based diets to poultry is constrained by the existence of anti-nutritional factors (ANFs) such as tannins, phytates and oxalates (Ogbe and Affiku, 2011). Unlike the seeds, the leaf contains a minute level of tannins and phytates but very free in trypsin inhibitors, amylase inhibitors, lectins, cyanogenic glucosides, and glucosinolates. Tannins inhibit the normal activity of protein enzymes in the gut by forming insoluble complexes with proteins, thereby making the protein contained in the ration inaccessible by the animals [7]. Additionally, tannins, phytates, and oxalates bind calcium and zinc in the feed and make them unavailable for absorption in the intestines (Ogbe and Affiku, 2011). Importantly, studies have proved that these ANFs are readily destroyed through soaking in water, fermentation technology or heat treatment (Igwilo *et al*., 2007).

Dietary moringa leaf meal has shown the ability to boost growth performance and immunity in broiler chickens (Liaqat *et al*., 2016) as they increase villi length in different parts of the intestine (Khan *et al*., 2017) that results to enhanced feed absorption (Awad *et al*., 2009). Improved fibre digestion in broilers fed moringa leaf product has been observed (Bustamante, 2014; Ustundag and Ozdogan, 2016). Scholars have disclosed that up to 15% MOLM can be added in broiler diet without negative impact on production parameters (Onunkwo and George, 2015). This report is in contradistinction with others (Bustamante *et al.,* 2013; Gadzirayi and Mupangwa, 2014 who observed poor performance data in terms of feed to gain ratio, weight gain and organ weight in broilers fed MOLM diet beyond 5% inclusion level. Incorporation of 1.2 g MOLM/kg feed in broiler feed resulted to an increased villus height when compared with the birds fed control diet (Khan *et al*., 2017). Hassan *et al*. (2016) noticed heavier organ and carcass weights in broilers fed containing MOLM at 1 g/kg feed and 3 g/kg feed suggesting that addition of MOLM at these levels of inclusions has no detrimental effect on the anatomy and physiology of chickens. A study by Cui *et al*. (2018) revealed that adding 15% MOLM in broiler feed raised the concentrations of linoleic, linolenic and arachidonic acids in breast meat and lowered the abdominal fat content. Interestingly, the introduction of 5% MOLM in chicken ration has helped to normalize the proportions of omega 6 and omega 3 fatty acids (FA) in broiler meat to an acceptable level for man's consumption by increasing the concentration of omega-3 FA while lowering the ratio of omega-6 to omega-3 FAs breast muscle (Nkukwana *et al*., 2014).

Currently, there is a paucity of information in the literature on optimizing MOLM as a nutritional strategy for improving the performance of broiler chickens. It is expected that this procedure will reduce wastage of vital feed components as well as optimize productivity. This will also help boost the socio-economic and nutritional standing of the farmers. Therefore, the objective of the present experiment was to determine the phytochemical composition of MOLM and the effect of MOLM supplementation levels on haemato-biochemical characteristics of Ross 308 broiler chickens. The optimal MOLM supplementation level that positively influenced the blood variables in Ross 308 broiler chickens aged from 1 to 42 days will also be modelled using the quadratic function.

4.2 Materials and Methods

4.21 Study location: The experiment was conducted between December 2017 and January, 2018 at broiler farm on Plot 519 in Zuurbekom, Westrand Region of Gauteng Province, South Africa. The environment temperature around the study location is ranged between 15 – 26^{o}C and 4 – 20^{o}C during the summer and winter respectively. The mean annual rainfall is in the study area is around 790 mm.

4.22 Experimental procedures

4.22.1 Preparation of the poultry house: The poultry house was thoroughly scrubbed, cleaned and disinfected with Virkon® S and later left for 7 days to dry**.** The poultry house was then left open for a week after cleaning so as to break the life cycle of any disease-causing organism that might have been missed by the disinfectant. The experimental house was divided into 25 floor pens of equal sizes of approximately **1.5/m^2** with 20 birds in each. Fresh sawdust was spread on the floor of the pens to a depth of 7 cm. Feeder and drinkers were thoroughly washed, disinfected with Virukill® before use. All the accessories required for the experiment were procured and assembled 5 days before the arrival of the broiler chicks. Heating equipment (infrared lights) were turned on 24 hours before the arrival of the chicks. Movement to the house was strictly restricted and footbath as a biosecurity measure was adopted throughout the experiment.

4.22.2 Preparation of MOLM and proximate analysis: Fresh moringa leaves were harvested in and around the Grootfontein village near Polokwane in Limpopo Province of South Africa. The leaves were air-dried at the normal room temperature for about 3 - 5 days, ground into a meal in a hammer mill and thereafter packaged and stored in polyethene bags for use in analysis and diet formulation. Proximate composition was determined in triplicates using a standard method (AOAC, 2000) at the Department of Animal Science Laboratory, North-West University-Mafikeng Campus, South Africa.

4.22.3 Experimental design, diet and ethics: A total of 500-day old unsexed Ross 308 broiler chicks were procured from National Chicks' Hatcheries located in Boschkop area East of Pretoria of Gauteng Province, South Africa. Five hundred-day old chicks of an average mean weight of 39.97 ± 0.23 g were randomly divided into five groups (TI, T2, T3, T4 and T5) of 100 chicks each with each group subdivided into 5 replicates of 20 birds each. Each group received a basal diet supplemented with MOLM at 0 g, 25 g, 50 g, 75 g and 100 g/kg feed, respectively for 42 days in a completely randomized design. The birds were fed were fed starter (1 - 21 days) and grower (22 - 42 days) diets (Table 4.1). Feed and water were provided *ad libitum* throughout the experiment, the light was provided 24 hours daily. The experimental birds were duly vaccinated. The birds were managed and raised under strict guidelines of the Ethics Committee of the University of South Africa (UNISA). The South African Animal Disease Act 35, of 1984 were highly taken into consideration.

4.23 Blood collection and analysis

At day 42 of the experiment, between the 7.00 h and 9.00h, 3 birds were randomly selected from each replicate, fasted for 6 h and blood samples were aspirated via the brachial vein. 7 ml of blood sample were drawn from each chicken using a scalp vein needle. Thereafter, 3 ml of the blood was transferred to the Ethylene-diamine tetra acetic acid (EDTA) treated vacutainer tube for haematological analysis; PCV, Hb, RBC, WBC, platelets, heterophil, lymphocytes, monocytes and basophils. The remaining 4 ml of blood sample was put into a non EDTA vacutainer tube for determination of serum biochemical parameters: total serum protein (TSP), albumin, glucose, uric acid, cholesterol, and triglyceride. The blood samples for analysis were taken the IDEXX Laboratories Johannesburg, South Africa immediately after collection following standard methods. The blood samples were analysed using the Anision - 3000 auto-analyzer system made by Pennyroyal.

4.24 Statistical analysis

Data collected were subjected to analysis of variance and where significant means were detected, the means were separated using Duncan's multiple range test (SPSS 2007). The dose related response in blood variables to MOLM supplementation were modeled using the quadratic optimization equation:

$Y = a + b_1x + b_2x^2$,

where

Y = blood variables;

a = the Y intercept;

b = coefficient of quadratic optimization equation;

x = MOLM supplementation levels and $-b_1/2b_2$ = x value for optimum response.

Table 4.1. Nutrient composition of experimental diets

Nutrient* (%)	Starter (0-21 days)	Grower (22 - 42 days)
Protein	21.0	19.0
Lysine	1.30	1.15
Methionine	0.58	0.5
Moisture	12.0	12.0
Fat	2.5	2.5
Fibre	5.0	6.0
Calcium	1.2	1.2
Phosphorus	0.6	0.55

*As illustrated in feed label

The quadratic equation was fitted to the experimental data by means of the nonlinear model procedure of SPSS (2007). A quadratic regression model was used because it fitted the model.

4.3 Results

Data on proximate composition of MOLM as shown in Table 4.2 revealed that crude protein (32.37%) was higher than 22.6 – 25.37% reported. The fibre composition of the MOLM was acid detergent fibre (4.68%) and neutral detergent fibre (52.16%).

Data on the influence of dietary MOLM supplementation on PCV, Hb, RBC, WBC and platelets of Ross 308 broiler chickens at 42 days of age are shown in Table 4.3. Mean PCV, Hb, RBC, WBC and platelets were 23.65%, 10.01 g/dl, 5.83 × 10^6/L, 21.32 × 10^6/L and 46.41 × 10^9/L, respectively while the coefficient of variations (CV) was ranged from 7.19 to 29.52%. Results showed that there was no significant ($p>0.05$) effect of MOLM supplementation on Hb concentration among the dietary groups. Birds fed diets supplemented with MOLM at 50 g/kg feed had higher ($p<0.05$) PCV and WBC count than those fed diet supplemental MOLM at 100 g/kg feed. There was significantly higher ($p<0.05$) RBC count for birds on 50 g/kg feed and 75 g/kg feed when compared with those on 0 g, 25 g and 100 g/kg feed. Birds offered diet supplemented with MOLM at 25 g/kg feed and 100 g/kg feed had the lowest blood platelet count which differed significantly from those on the other three diets.

Results of the differential WBC counts of broilers on supplemental inclusion level of MOLM are presented in Table 4.4. Mean lymphocyte, heterophil, monocytes and basophils were 44.79%, 35.09%, 7.82% and 0.002%, respectively. The CV for the differential WBC was ranged from 20.35 to 200%. Heterophil and monocyte achieved the same pattern as the supplementation level of MOLM were increased in the diets with values from birds fed MOLM at 50 g/kg feed being higher ($p<0.05$) than those on 25 and 100 g MOLM/kg feed. Chickens fed control diet had higher ($p<0.05$) lymphocyte than those on 100 g MOLM/kg feed but similar to those on 25, 50 and 75 g MOLM/kg feed. Basophil count of the control birds was higher ($p<0.05$) compared to those birds on treatment groups.

Table 4.2. Chemical Composition of MOLM

Bioactive compounds	Composition (%)
Moisture	7.83
Crude protein	32.37
Ether extract	2.09
Crude fibre	3.10
Acid detergent fibre (ADF)	4.68
Neutral detergent fibre (NDF)	52.16
Ash	5.94

Table 4.3. Haematological values of Ross 308 broilers fed MOLM supplemented rations

MOLM Level	PCV (%)	Hb (g/dl)	RBC (x10^6/L)	WBC (x10^3/L)	Platelets (x10^9/L)
0 g/kg feed	35.58^{bc}	10.24	6.85^{a}	21.44^{ab}	51.48^{cd}
25 g/kg feed	29.86^{ab}	10.21	6.54^{a}	21.91^{ab}	29.29^{a}
50 g/kg feed	40.17^{c}	11.56	8.91^{b}	25.01^{b}	47.86^{bc}
75 g/kg feed	40.07^{c}	11.54	8.86^{b}	21.90^{ab}	65.38^{d}
100 g/kg feed	23.65^{a}	10.01	5.83^{a}	16.33^{a}	38.03^{ab}
Mean	33.87	10.71	7.40	21.32	46.41
SD	7.10	0.77	1.41	3.13	13.70
CV (%)	20.96	7.19	19.05	14.68	29.52
SEM	3.17	0.34	0.63	1.40	6.13
p-value	0.03	0.52	0.08	0.2	0.04

a-c means with the same letters within column differed statistically at $p<0.05$, SD - standard deviation, CV- coefficient of variation, SEM – standard error of the mean.

Table 4.4. Differential white blood cell value of Ross 308 broilers fed MOLM based rations

MOLM level	Lymphocyte (%)	Heterophil(%)	Monocyte (%)	Basophil (%)
0 g/kg feed	57.67^{b}	34.86^{ab}	7.39^{ab}	0.01^{b}
25 g/kg feed	42.40^{b}	30.67^{a}	5.50^{a}	0.00^{a}
50 g/kg feed	45.67^{b}	40.63^{c}	13.70^{b}	0.00^{a}
75 g/kg feed	52.70^{b}	39.00^{bc}	8.30^{ab}	0.00^{a}
100 g/kg feed	29.53^{a}	26.27^{a}	4.20^{a}	0.00^{a}
Mean	44.79	35.09	7.82	0.002
SD	10.92	7.14	3.66	0.004
CV (%)	24.38	20.35	46.80	200.00
SEM	4.88	3.19	1.64	0.002
p-value	0.015	0.046	0.035	0.042

a-c means with the same letters within column differed statistically at $p<0.05$, SD- standard deviation, CV- coefficient of variation, SEM – standard error of the mean.

Mean TSP, albumin, glucose, triglycerides, cholesterol and uric acid values of Ross 308 fed diets containing varying supplementation levels were 3.66 g/dl, 1.28 g/dl, 240.34 mg/dl, 14.22 mg/dl, 74.63 mg/dl and 0.30 µmol/l respectively (Table 4.5). The coefficient of variations (CV) ranged from 1.09 to 18.35% with TSP having the least dispersion while triglycerides recorded the highest dispersion. Ross 308 broilers diets supplemented with MOLM at 0, 25, 50, 75 and 100 g/kg feed had similar ($p>0.05$) TSP and albumin values. Broiler chickens on 50 and 75 g MOLM/kg diet had higher ($p<0.05$) serum glucose value than those fed diets with MOLM at 0, 25 and 100 g/kg diet. Chickens on MOLM supplemented diets had higher serum cholesterol value than those fed diet with MOLM at 0 g/kg feed. However, the observed difference was not significant ($p>0.05$). There were also no statistical differences ($p<0.05$) in serum uric acid values across treatment levels.

MOLM supplementation levels had a quadratic influence on some aspect of blood values of Ross 308 broilers (Table 4.6). MOLM was observed to have a quadratic influence on optimal PCV with a quadratic value of $22.056 + 12.268$ MOLM - 2.2721 MOLM^2, r2 = 0.451 with the optimum MOLM supplementation level being 26.99 g/kg feed. Similar quadratic effects were noticed for serum glucose ($219.96 + 15.938$ MOLM - 2.49431MOLM^2, $r^2 = 0.239$ with the optimum MOLM supplementation rate being 31.99 g/kg feed). However, this level was higher than 26.99 g/kg feed needed for optimizing the PCV. The quadratic response of PCV and glucose value of Ross 308 broilers to MOLM supplementation are presented in Fig. 4.1 and 4.2, respectively.

Table 4.5**.** Serum biochemical values of Ross 308 broilers on MOLM supplemented diets

MOLM level	TSP (g/dl)	Albumin (g/dl)	Glucose (mg/dl)	Triglycerides (mg/dl)	Cholesterol (mg/dl)	Uric acid (µmol/l)
0 g/kg feed	3.68	1.23	238.32[a]	18.18[b]	70.02	0.27
25 g/kg feed	3.61	1.27	228.60[a]	11.70[a]	76.14	0.25
50 g/kg feed	3.69	1.34	255.60[b]	15.48[b]	73.08	0.37
75 g/kg feed	3.62	1.28	243.36[b]	12.96[a]	75.42	0.30
100 g/kg feed	3.69	1.29	235.80[a]	12.78[a]	78.48	0.31
Mean	3.66	1.28	240.34	14.22	74.63	0.30
SD	0.04	0.04	10.06	2.61	3.22	0.04
CV (%)	1.09	3.13	4.19	18.35	4.31	13.33
SEM	0.02	0.02	0.50	1.17	1.44	0.02
p-value	0.99	0.95	0.15	0.75	0.64	0.75

a-b means with the same letters within column differed statistically at $p<0.05$, SD- standard deviation, CV- coefficient of variation, SEM – standard error of the mean.

Table 4.6**.** Dose response effect of MOLM supplementation on PCV and glucose level of Ross 308 broilers

Blood variables	R^2	Optimal MOLM level	Optimal Y level	Probability
PCV (%)	0.451	26.99	38.62	0.043
Glucose (mg/dl)	0.239	31.95	240.54	0.035

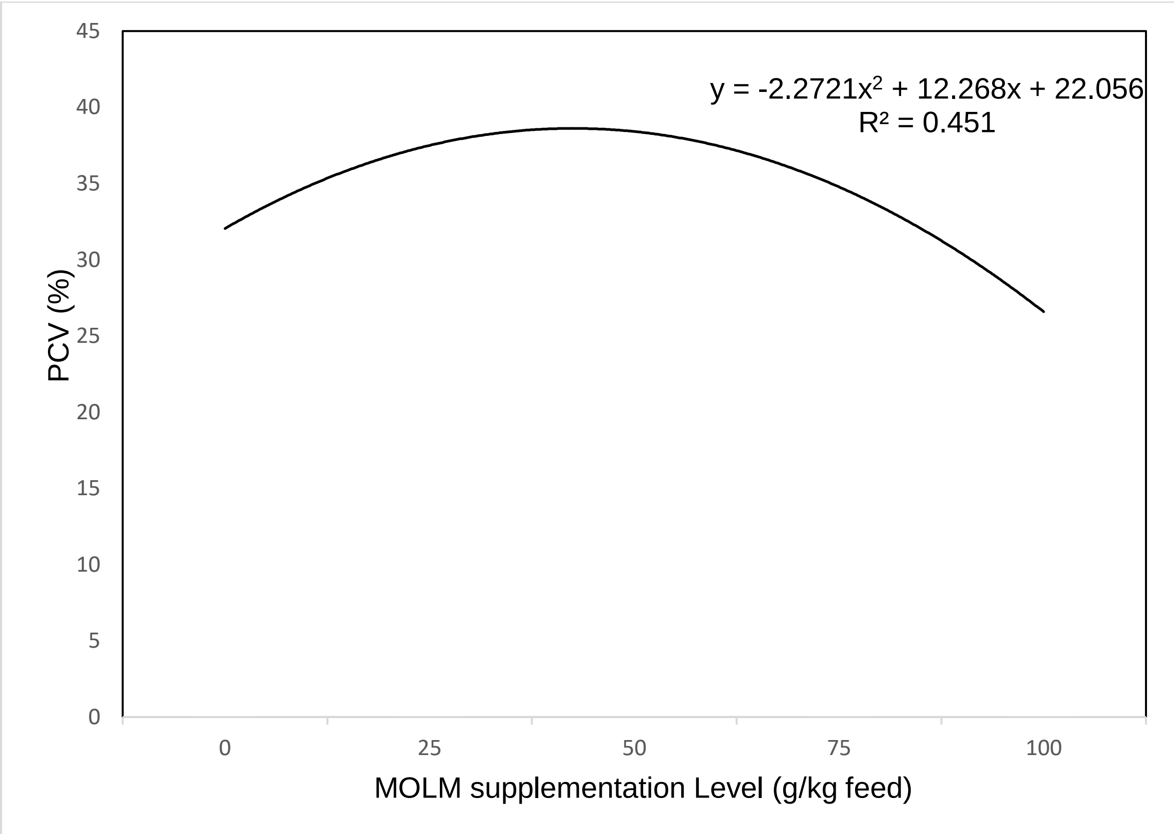

Fig. 4.1. Effect of MOLM supplementation level on optimum PCV level in Ross 308 broilers

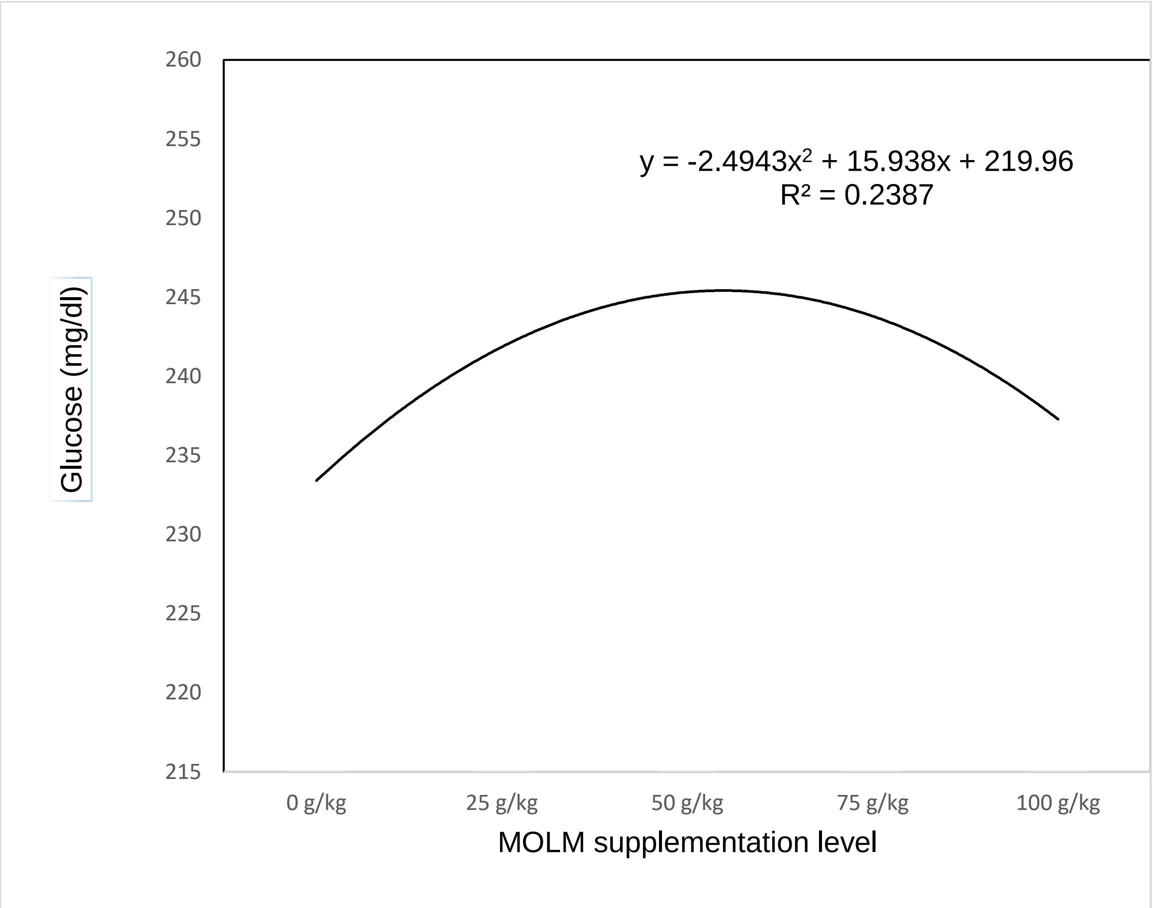

Fig. 4.2. Effect of MOLM supplementation level on optimum glucose level in Ross 308 broilers

4.4 Discussion

Data on proximate composition of MOLM revealed that crude protein (32.37%) was higher than 22.6 – 25.37% reported by others (Tijani *et al*., 2015; Alnidawi *et al*., 2016; Alabi *et al*., 2017). The ash value (5.94%) was similar to the value 5.89% (Alnidawi *et al*., 2016) but lower than 7.80 – 9.75% reported by Tijani *et al*. (2015) and Alabi *et al*. (2017), while the ether extract content (2.09%) was slightly lower than the range of 2.44 – 5.50% obtained by other investigators (Tijani *et al*., 2015; Alabi *et al*., 2017). The crude fibre content (3.10%) was more than four folds lower than the values of 16.57% and 17.41% reported by Tijani *et al*. (2015) and Alabi *et al*. (2017), respectively and more than four folds lower than 10.10% earlier obtained by Tijani *et al*. (2015). The fibre composition of the MOLM was acid detergent fibre (4.68%) and neutral detergent fibre (52.16%).

The results of the current feeding trial revealed that Ross 308 broilers fed MOLM at 50 and 75 g/kg feed had comparable PCV values with those on 0 and 25 g MOLM/kg feed, but higher than the values (29.91 – 31.79% and 16.67 – 23%) observed in broilers fed MOLM at 50 - 200 g/kg feed and 100 – 200 g/kg feed (Tijani *et al*., 2015). However, supplementation to the rate of 100 g/kg feed leads to significant decline in PCV. This observation is in disagreement with (Onu and Aniebo, 2011), who reported increased PCV in broilers fed MOLM at 100 g/kg feed. The observed decline in PCV beyond 75% as reported in the current feeding trial may be linked to the anti-physiological agents present in moringa leaves (Alabi *et al*., 2017), which may have exceeded the limit the birds could handle physiologically. With the exception of Ross 308 broilers on 25 and 100 g MOLM/kg feed, the PCV at the other 3 treatments were within the reference value (30 - 40%) reported by Aiello and Mays (1998) for clinically healthy chickens. Additionally, the abnormally low PCV of Ross 308 broilers on diet contained MOLM at 25 and 100 g/kg feed may indicate anaemia, a reduction in total red blood cell count. The Hb concentration in all the five treatments was within the standard range (9 – 13 g/dl) reported for healthy chickens in the literature (Aiello and Mays, 1998). The increased Hb and RBC count of birds fed a diet containing MOLM at 50 and 75 g/kg feed may be attributed to enhanced utilisation of the diets by the treatment birds (Onu and Aniebo, 2011). However, the RBC values (5.83 – 8.91 × 10^6/L) recorded for broilers in the present study were higher than the normative count of 3 × 10^6/L reported for chickens in the temperate region (Aiello and Mays, 1998). The observed slight polycythaemia could be attributed to a decline in blood plasma because of insufficient water intakes by the experimental birds. Moringa leaf meal as in any other forages is abundant in fibre content that may lead dehydration because of insufficient water intake by the broilers for normal physiological activities. Our results revealed that MOLM based diets significantly influenced platelet count with the Ross 308 broilers on 75 g/kg feed having the highest count. This finding is in contrast with Alnidawi *et al*. (2016) who observed a slight reduction in platelet count in broilers fed the same level of MOLM for 42 days. The disparity may be attributed to the chemical composition of the test leaf meal as well as the physicochemical of the diet.

Examination of the results of the WBC counts showed that broilers fed a diet containing MOLM at 50 g/kg feed was statistically higher than the broilers on 100 g MOLM/kg feed. However, the significant decrease in the WBC count of broilers on MOLM supplementation rate of 100 g/kg feed suggests the possibility of the toxicity of diet (Tijani *et al*., 2015). This result supported (Gupta *et al*., 2010) who noticed that moringa leaf extract has an immunomodulatory action in small laboratory animal model. This decline in WBC may have been triggered by high levels of anti-physiological agents in the diet with MOLM at 100 g/kg feed. The results of the present experiment revealed that MOLM supplementation had significant effects on differential WBC counts in Ross 308 broiler chickens. The observed significant decline in the differential WBC counts (lymphocytes, heterophils and monocytes) at 100 g/kg feed may again be due to high levels of anti-physiological agents in the diets that translate to poor nutrient utilization by the experimental chickens. The decrease in heterophil count of Ross 308 broilers on 100 g/kg feed contrasted with Tijani *et al*. (2015), who noticed slightly increased heterophil count in Anak broilers fed a diet containing leaf meal of *Moringa oleifera* plant at 100 g/kg feed for 56 days in southwest Nigeria. The observed difference may be due to environmental and soil type differences, which has been observed to influence phytochemical and nutrient composition of *Moringa oleifera* leaves (Melesse *et al*., 2012; Kumssa *et al*., 2017).

The blood biochemical results revealed no significant variation in the TSP, albumin, cholesterol and uric acid value of Ross 308 broilers on 25, 50, 75 and 100 g MOLM/kg feed, thus showing that MOLM supplementation had no adverse impacts on these parameters investigated. However, MOLM supplementation had a significant impact on serum glucose level among the birds. The groups fed a diet containing MOLM at 50 and 75 g/kg feed diet recorded higher serum glucose than those on the other three treatments. At present, there is no published study in the literature on the effect of moringa leaves on blood glucose level of chickens. However, a study conducted by Edoga *et al*. (2013) in rats showed that moringa leaves have hypoglycaemic effect in rats. The disparity spotted in these two studies may be ascribed to species difference, the age of the animals and duration of feeding the test leaves. The observed reductions in the serum triglyceride concentration corroborates (Edoga *et al*., (2013), who discovered that MOLM has hypolipidemic effect on chickens. However, the observed 12.08% increase in serum cholesterol level of birds fed MOLM at 100 g/kg feed disagreed with the findings of other researchers (Divya *et al.,* 2014; Alnidawi *et al*. 2016)) who reported that *Moringa oleifera* leaf meal has antihypercholesterolemic effects on broiler chickens. Furthermore, studies (Alnidawi *et al*. (2016) have shown that feeding *Moringa oleifera* leaf meal to broilers reduces the concentrations of the low-density cholesterol (LDL-c) and increases the concentrations of high-density cholesterol (HDL-c). Thus, the observed increase in serum cholesterol in Ross 308 broilers fed MOLM at 50 g/kg feed and 75 g/kg feed may be an indication that a greater proportion of the cholesterol are HDL as reported earlier by Alnidawi *et al*. (2016). Uric acid is the last point of purine and protein metabolism in avian species, and they are produced in the liver and excreted by the kidney. However, in a state of negative energy balance that usually occurred when birds rely on proteins to meet up their daily energy need, the concentration of uric acid tends to accumulate in the blood, especially when it is beyond the level the kidney can handle. The comparable serum uric acid levels among the treatments ruled out the possibility of excessive protein breakdown thus showing that the energy level of the experimental diets is not compromised. This is also supported by the serum glucose and protein results in the current study.

The results of the current experiment reveal that MOLM supplementation had a quadratic effect on serum glucose and PCV with a probability value of 0.239 to 0.451, respectively. The coefficient of determination value of 23.9% to 45.1% recorded in this study suggest that there is a low to moderate effect of MOLM supplementation on serum glucose and PCV in Ross 308 broilers using quadratic optimization function. The PCV and serum glucose were observed to be optimized at different supplementation levels and the reason for the disparity is not known. However, it may be that these blood parameters require different dietary components and levels for their production. Thus, in agreement with Mbajiorgu (2011) and Mbajiorgu *et al*. (2011) who observed that optimum dose response value of dietary nutrients inclusion levels for optimizing different parameters in chickens is dynamic. The optimum MOLM level of 38.62 g/kg feed that optimized PCV was higher than 29.93, 25.22, 28.81 and 26.22 g/kg feed reported by Liaqat *et al.* (2016), Alnidawi *et al*. (2016), Makanjuola *et al*. (2014) and Safa and Ibrahim (2014), respectively in broiler chickens subjected to different supplementation levels of MOLM. However, the PCV of 26.99% recorded in the present study was lower than 30.09 - 31.54%

reported in the literature (Safa and Ibrahim, 2014; Makanjuola *et al*., 2014; Alnidawi *et al*., 2016). The reason for these differences in requirements is not known, however, it is possible that such discrepant variation in requirement may be due to alterations in supplemental MOLM inclusion level which allowed for less equilibrium in blood quantity and quality leading to a difference in requirement. Continued study of the spectrum of MOLM inclusion level in blood traits in Ross 308 broilers is desirable.

4.5 Conclusion

From our results, it is concluded that *Moringa oleifera* leaf meal is high in protein and mineral content and is suitable for use in broiler ration. Hence, moringa leaves can be utilized as an excellent protein source in broiler production. MOLM supplementation levels of 26.99% and 31.95% respectively are needed to optimize packed cell volume (36.882%) and serum glucose (240.55 mg/dl) in Ross 308 broilers. Thus, optimizing MOLM supplementation in the ration of Ross 308 broilers could assist in improving health status and physiological wellbeing of chickens.

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CHAPTER 5

GENERAL CONCLUSION AND RECOMMENDATIONS

5.1 General conclusions

MOLM is a good source of protein and mineral (calcium, sodium, potassium, iron and sulphur) and can be utilized as an excellent protein and mineral sources in broiler production. This is also an indication that *Moringa oleifera* tree has the potential to take up and store beneficial minerals in its leaves. The high coefficient of variation of mean mineral values recorded in the present experiment showed that these means could not serve as reference mineral value for MOLM in the study region.

MOLM significantly influenced live weight and average daily gain on broilers at 21 days of age. Increasing MOLM supplementation in broiler at 21 days of age resulted in poor feed conversion ratio, hence negatively affecting live weight and average daily gain of broilers at 21 days of age. On the other hand, increasing MOLM supplementation reduced live weight in broilers at 42 days of age without adversely affecting feed intake, average daily gain and feed conversion ratio. Mortality rate was significantly lower in both starter and finisher broilers that received varying supplementation levels of MOLM when compared with birds that received control diet. This shows that MOLM is rich in bioactive ingredients, which have been reported in the literature to have medicinal and pharmacological properties in animals.

MOLM supplementation supported carcass weight and dressing percent at 25, 50, 75 and 100 g/kg feed supplementation. The comparable carcass weight (breast, drumstick and thigh) and organ weights (liver and heart) when compared with the broilers that received control diet suggested that MOLM supplementation positively influenced the development of the cut parts without negatively affecting the organ functions.

MOLM supplementation influenced haematological indices (PCV, Hb, RBC, WBC and blood platelets) in broilers at 42 days of age. MOLM supplementation at the rates of 25, 50, 75 and 100 g MOLM/kg feed in Ross 308 diets supported the production of total serum protein, albumin, cholesterol and uric acid value. However, MOLM supplementation at 50 and 75 g/kg feed increased serum glucose level.

The results of the quadratic function showed that no single MOLM supplementation level optimized FLW, ADG, PCV and glucose in the current feeding experiment. Thus, the present investigation indicated that a diet containing MOLM levels at 39.98 and 35.80 g/kg feed allowed for optimal utilization of absorbed nutrients for growth in Ross 308 broilers aged day old to 21 days. FLW, PCV and glucose were optimized in Ross 308 broilers at 42 days of age at 46.88, 26.99 and 31.95 g/kg feed inclusion levels, thus showing that the dietary MOLM supplementation level for optimal response is achieved below 50 g/kg feed supplementation.

5.2 Recommendations

Dietary MOLM level for optimal response in Ross 308 broilers was not achieved at a single supplementation level. This may imply that the MOLM supplementation level needed to improve broilers performance is dynamic and dependent on the production variable is taken into consideration when compounding the diets. Thus, the feeding program for optimal production in Ross 308 broilers fed MOLM supplemented diets must take into consideration the key variable in question. MOLM is a good source of vital nutrients and is suitable for cut parts development and production of enhanced cut parts in the broiler chickens. It is therefore, recommended that supplementation rate of up to 100 g/kg feed MOLM may be well accepted by the Ross 308 broiler chickens. More research is needed to fully understand the impacts of dietary MOLM on the histoarchitecture of the organs and intestines of Ross 308 broilers. The application of transcriptomics technologies in the explanation of the actual mechanisms behind the health benefits of MOLM in broiler production is strongly advocated because for now there is no such information in the literature

APPENDIX

PUBLICATIONS FROM THE THESIS:

1. **Morategi Modisaojang-Mojanaga, I.P. Ogbuewu, J.W Oguttu and C.A Mbajiorgu.2019.** Moringa leaf meal improves haemato-biochemical and production indices in broiler chickens: a review: **Comparative Clinical Pathology;** ISSN: 1618-5641(Print) 1618-565X (Online). **(Published online first 13th January 2019).**

2. **Morategi Modisaojang-Mojanaga, I.P. Ogbuewu, J.W Oguttu and C.A Mbajiorgu**. **2019.** Moderate levels of dietary Moringa oleifera leaf meal improves blood values in broiler chickens**: Journal of food, Agriculture & Environment:** Electronic/online ISSN; 1459-0263(Print). **(Accepted 11 December 2018.)**

3. **Modisaojang-Mojanaga, M.M.C. - Ogbuewu, I.P*. - Mokolopi, B.G. - Oguttu, J.W. - Mbajiorgu, C.A. 2019**. MINERAL COMPOSITION OF MORINGA OLEIFERA LEAF MEAL (MOLM) AND THE RESPONSES OF ROSS 308 BROILERS TO MOLM SUPPLEMENTATION**. Applied Ecology and Environmental Research**. **(Under review).**

Comparative Clinical Pathology
https://doi.org/10.1007/s00580-019-02900-7

Moringa leaf meal improves haemato-biochemical and production indices in broiler chickens: a review.

M. M. Modisaojang-Mojanaga1 & I. P. Ogbuewu1 & J. W. Oguttu1 & C. A. Mbajiorgu1

Received: 23 October 2018 /**Accepted:** 3 January 2019

Abstract
The high cost of feed materials and feed additives in developing nations has elicited interest in the search for sustainable alternatives having in mind the human food security. Moringa (*Moringa oleifera*), one of such sustainable alternatives, is a tropical plant with excellent nutritive and phytochemical content. It is one of the species in the family of Moringaceae and thrives well in the tropics. The excellent nutritional quality of *M. oleifera* has positioned it as a choice feed ingredient/additive for broiler production. Furthermore, the high carotenoid content of moringa leaves offers great potential for its use in pigmentation of egg yolk and broiler meat. Moringa has antibacterial, antifungal, anti-inflammatory, antihypercholesterolemic and antioxidant properties. Besides the nutritional and phytochemical value of this plant, their use as a feed supplement or feedstuff and their beneficial attributes on production indices and heamato-biochemical values of broiler chickens are also discussed. Alongside their excellent chemical composition, this paper also tends to showcase the plant as a promising novel feedstuff/additive source in broiler nutrition. The use of moringa in broiler nutrition as a novel feed additive and nutrient source will not only reduce feed cost but will also improve production performance, ensure healthy animal products and a better environment.

Keywords: Production performance. Blood. Broiler chickens. Moringa leaf meal
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Journal of Food, Agriculture & Environment (JFAE)

Letter of Acceptance **30.11.2018**

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Moderate levels of dietary *Moringa oleifera* leaf meal supplementation improves blood values in broiler chickens.

Moratégi M.C. Modisaojang-Mojanaga, Ifeanyi P. Ogbuewu*, James W. Oguttu and Christian A. Mbajiorgu.

Department of Agriculture and Animal Health, University of South Africa, Florida Science Campus, Johannesburg, South Africa
**e-mail: dr.ogbuewu@gmail.com; ogbueip@unisa.ac.za*

Your manuscript has been accepted for publication in the Electronic scientific Journal of Food, Agriculture & Environment *JFAE Vol. 17 (1) January 2019.* Our team has made some minor corrections **but** you must improve and correct the galley proof accurately before sending it back to us (ask help from a native English speaker or scientist). Your manuscript is scheduled to appear in the *JFAE Vol. 17 (1) January 2019.*

MINERAL COMPOSITION OF MORINGA OLEIFERA LEAF MEAL (MOLM) AND THE RESPONSES OF ROSS 308 BROILERS TO MOLM SUPPLEMENTATION

Modisaojang-Mojanaga, M.M.C. - Ogbuewu, I.P*. - Mokolopi, B.G. - Oguttu, J.W. - Mbajiorgu, C.A.
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Private Bag X6, Florida 1710, Johannesburg, South Africa.
**Corresponding author's e-mail: dr.ogbuewu@gmail.com; ogbueip@unisa.ac.za*

Abstract. A 42-day study was designed to determine the mineral composition of MOLM and the response of Ross 308 broilers to dietary MOLM supplementation. Day-old Ross 308 broiler chicks (n = 500) were allotted to five treatments in completely randomized design with each group replicated five times with each replicate having 20 chicks. Birds received diets supplemented with MOLM at 0, 25, 50, 75 and 100 g/kg feed, respectively and assigned T1, T2, T3, T4 and T5. MOLM level that supported optimum variables was modelled using the quadratic function. At day 42, three birds per replicate were slaughtered to evaluate carcass and organ yields. Result of mineral assay indicate that MOLM was high in calcium, sodium, potassium, sulphur and iron. Daily feed intake (FI), average daily gain (ADG) and feed conversion ratio were the same among the groups with the exception of starter broilers on diet T1 that had higher ADG ($p<0.05$) than those on the other diets. Final live weight (FLW), mortality and gizzard weight were influenced ($p<0.05$) by MOLM supplementation. MOLM supplementation had no effect on parameters measured. MOLM supplementation at 39.98 and 35.80 g/kg feed supported optimum FLW and ADG at starter phase and 46.88 g/kg feed MOLM supported optimum FLW at finisher phase. In conclusion, MOLM is a good source of nutrients and suitable for production of enhanced cut parts in broiler chickens.

Keyword: *alternative feedstuffs, broilers, optimization, production outputs, South Africa*

UNISA university of south africa

CAES ANIMAL RESEARCH ETHICS REVIEW COMMITTEE

Date: 03/03/2017

Ref #: **2017/CAES/028**
Name of applicant: **Ms MMC Mojanaga**
Student #: **58563792**

Dear Ms Mojanaga,

Decision: Ethics Approval

Proposal: Physiological responses of Ross 308 broiler chickens fed graded levels of *Moringa oleifera* leaf meal (molm): some aspects of haematology and serum biochemistry

Supervisor: Prof C Mbajiorgu

Qualification: Postgraduate degree

Thank you for the application for research ethics clearance by the CAES Animal Research Ethics Review Committee for the above mentioned research. Approval is granted for the project, *subject to submission of a signed indemnity agreement between the researcher and farm owner covering the handover of the chickens at the end of the project.*

Please note that the approval is valid for a one year period only. After one year the researcher is required to submit a progress report, upon which the ethics clearance may be renewed for another year.

Due date for progress report: 31 March 2018

Please note points 4 and 5 below for further action.

The application was reviewed in compliance with the Unisa Policy on Research Ethics by the CAES Animal Research Ethics Review Committee on 02 March 2017.

The proposed research may now commence with the proviso that:

1) *The researcher/s will ensure that the research project adheres to the values and principles expressed in the UNISA Policy on Research Ethics.*

University of South Africa
Preller Street, Muckleneuk Ridge, City of Tshwane
PO Box 392 UNISA 0003 South Africa
Telephone: +27 12 429 3111 Facsimile: +27 12 429 4150
www.unisa.ac.za

Vaccination program used for the study were indicated below:

Day one (On arrival)

Chicks were vaccinated against Newcastle disease from the hatchery, Vita stress was added to their drinking water on arrival for the first two days to calm them down from handling and transportation stress.

Day three

Tylotad was added in the drinking water for prevention of *Escherichia coli (E. coli)* bacteria and other disease-causing microorganisms.

Day seven

Chicks were vaccinated against Infectious Bronchitis using “IBH 120”.

Day twelve

Chicks were vaccinated against Gumboro through drinking water.

Day eighteen

Re-vaccination against Gumboro.

Day twenty - one

Tylotad was added to their drinking water whereby the withdrawal period will be 15 days.

Day twenty – three

Chicks were given Newcastle vaccine booster.

The GLM Procedure

Class Level Information

Class	Levels	Values
treat	5	A B C D E

Number of Observations Read	25
Number of Observations Used	25

The GLM Procedure

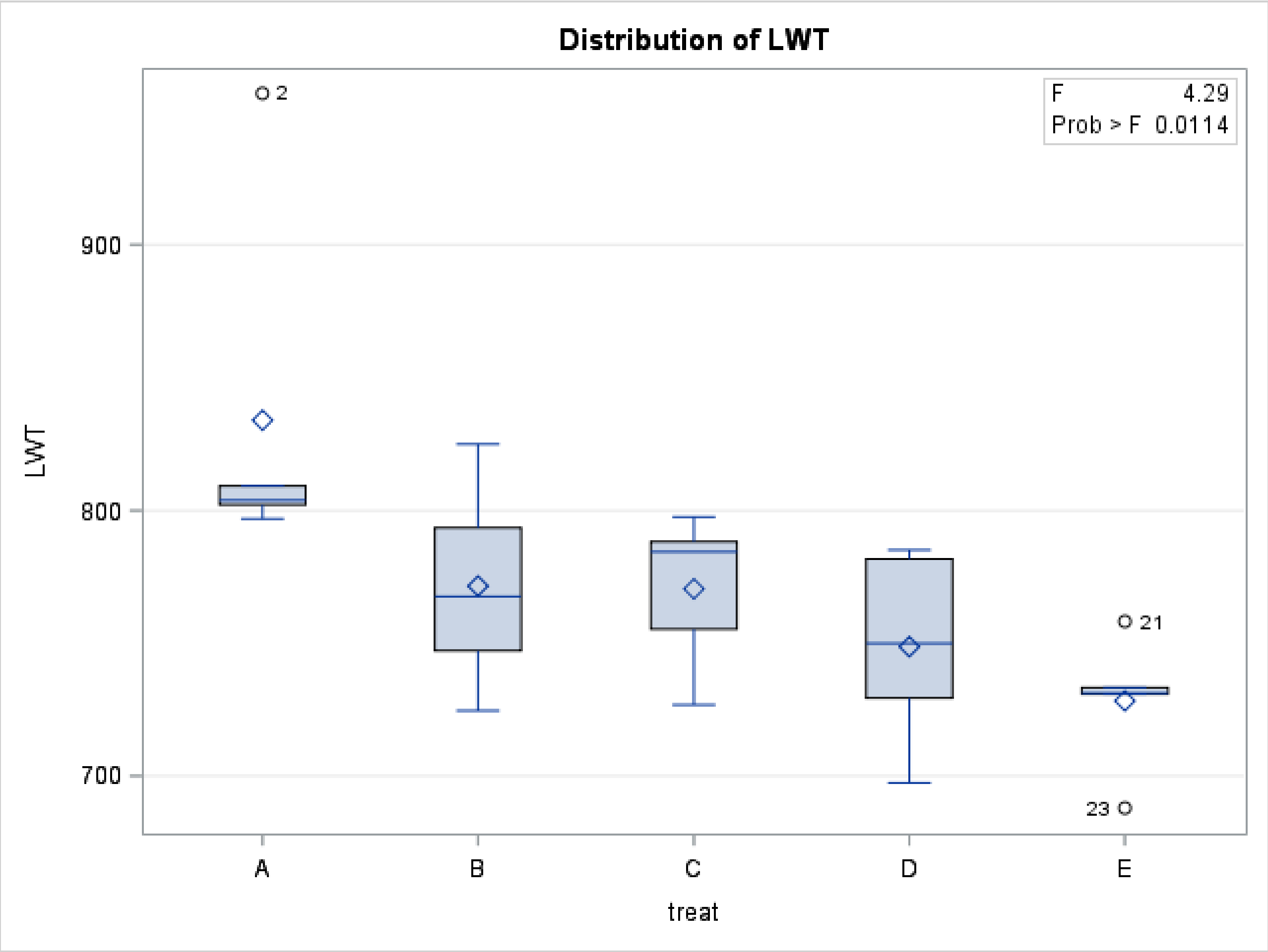
Dependent Variable: LWT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	31361.51706	7840.37926	4.29	0.0114
Error	20	36542.89668	1827.14483		
Corrected Total	24	67904.41374			

R-Square	Coeff Var	Root MSE	LWT Mean
0.461848	5.547075	42.74511	770.5884

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	31361.51706	7840.37926	4.29	0.0114

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	31361.51706	7840.37926	4.29	0.0114



The SAS System

The GLM Procedure

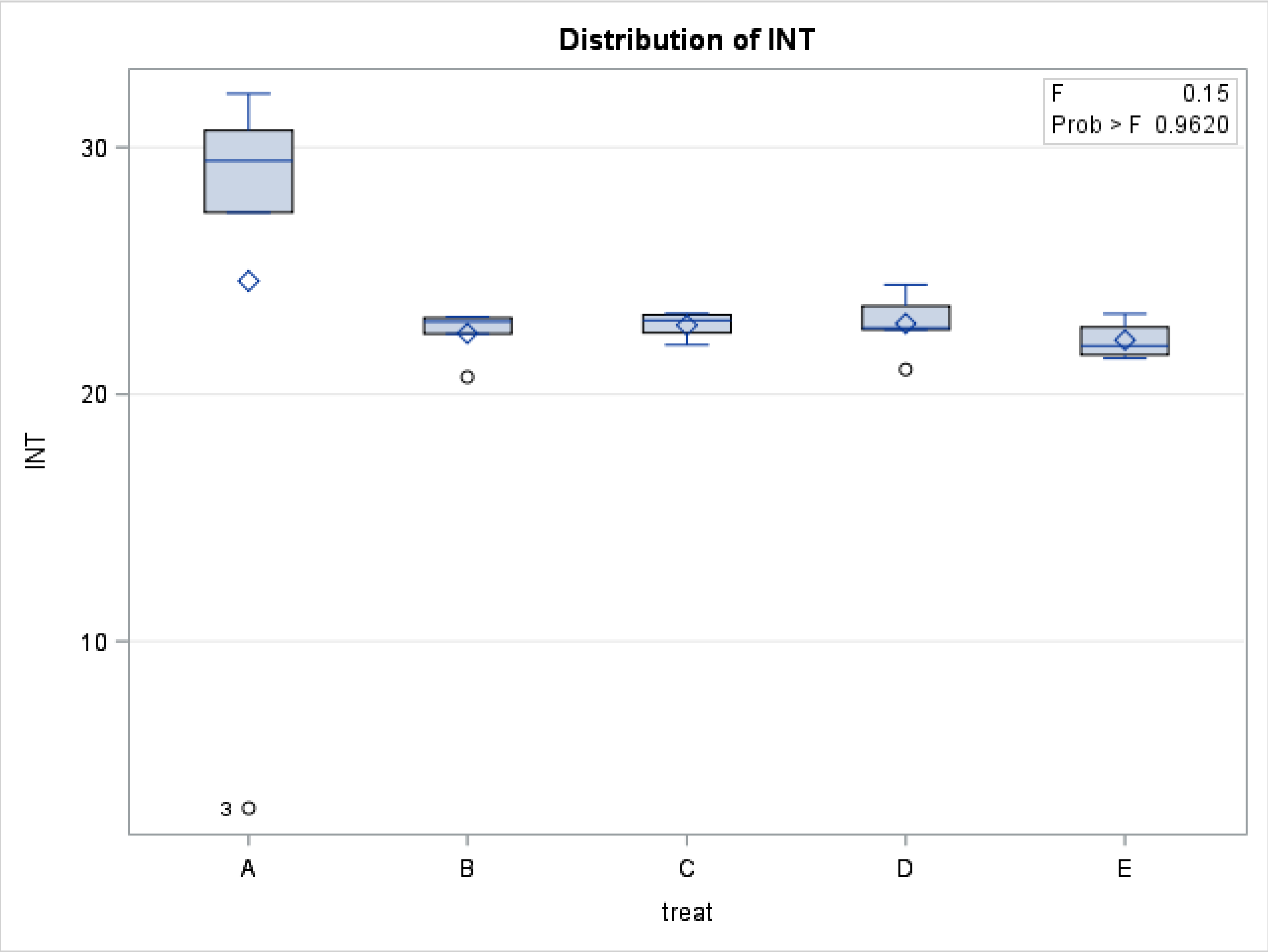
Dependent Variable: INT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	17.5851040	4.3962760	0.15	0.9620
Error	20	596.2425200	29.8121260		
Corrected Total	24	613.8276240			

R-Square	Coeff Var	Root MSE	INT Mean
0.028648	23.75463	5.460048	22.98520

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	17.58510400	4.39627600	0.15	0.9620

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	17.58510400	4.39627600	0.15	0.9620



The GLM Procedure

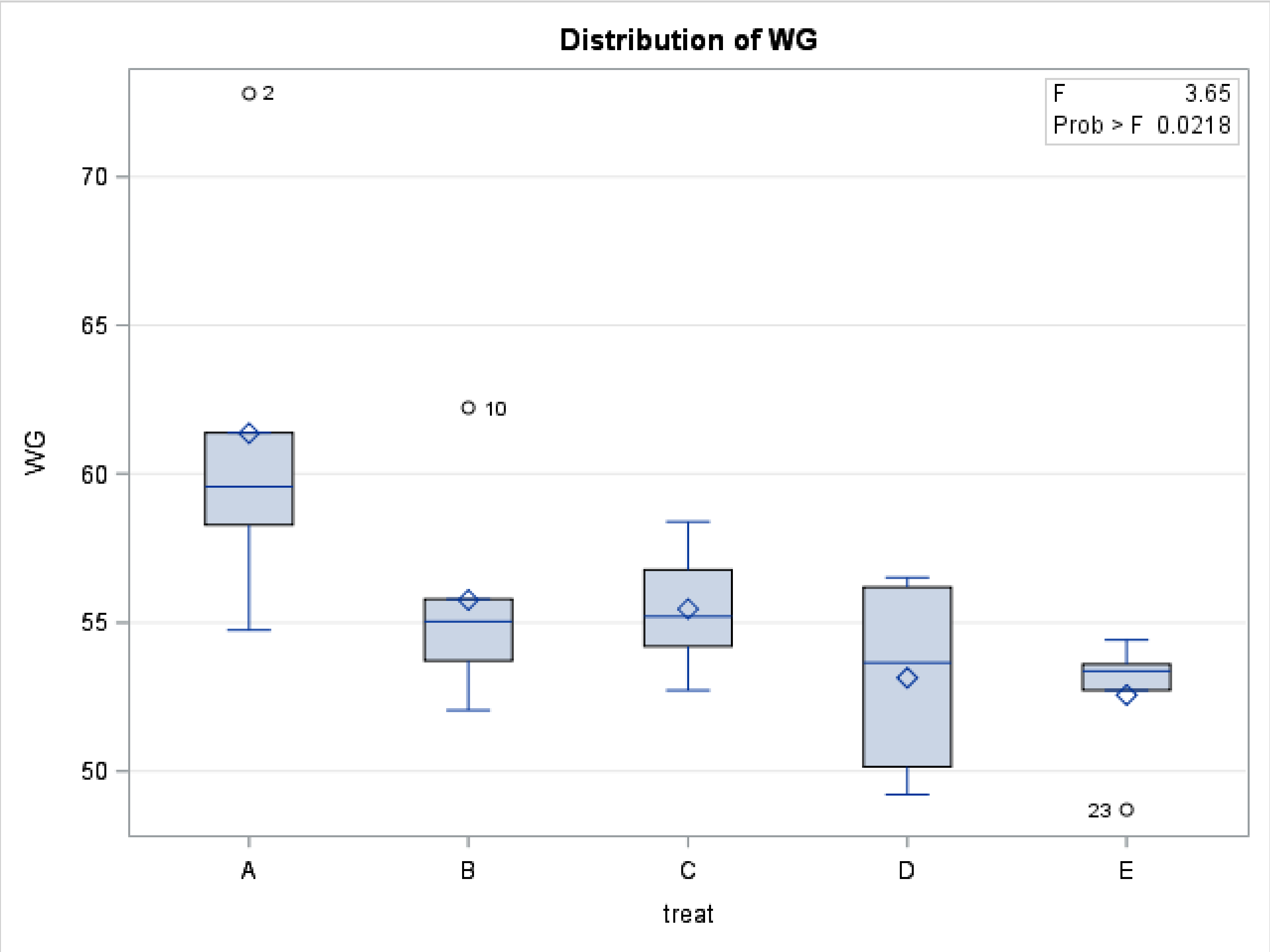
Dependent Variable: WG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	242.3393360	60.5848340	3.65	0.0218
Error	20	332.3740000	16.6187000		
Corrected Total	24	574.7133360			

R-Square	Coeff Var	Root MSE	WG Mean
0.421670	7.324328	4.076604	55.65840

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	242.3393360	60.5848340	3.65	0.0218

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	242.3393360	60.5848340	3.65	0.0218



The SAS System

The GLM Procedure

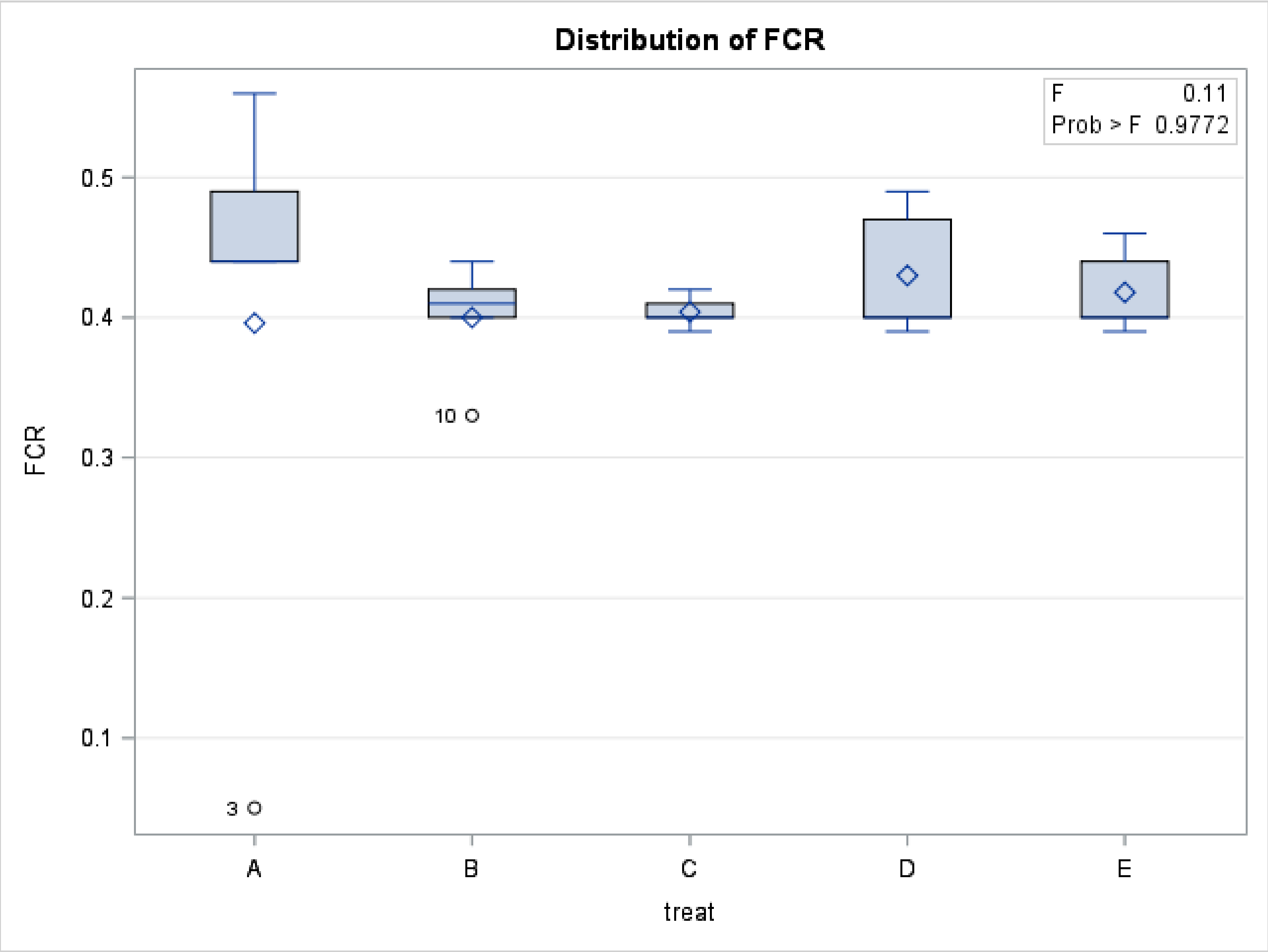
Dependent Variable: FCR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.00397600	0.00099400	0.11	0.9772
Error	20	0.17912000	0.00895600		
Corrected Total	24	0.18309600			

R-Square	Coeff Var	Root MSE	FCR Mean
0.021715	23.10453	0.094636	0.409600

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	0.00397600	0.00099400	0.11	0.9772

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	0.00397600	0.00099400	0.11	0.9772



The GLM Procedure

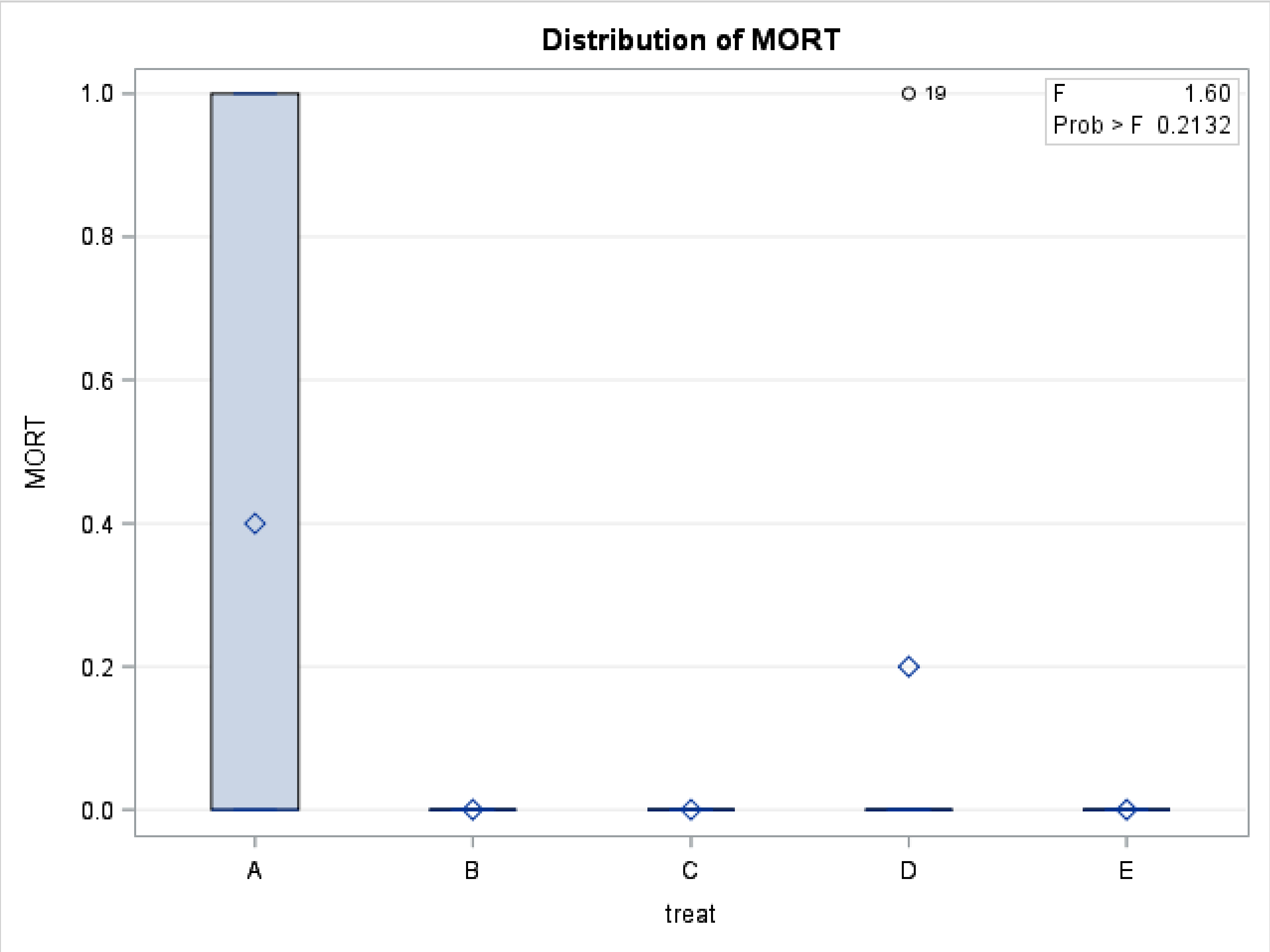
Dependent Variable: MORT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.64000000	0.16000000	1.60	0.2132
Error	20	2.00000000	0.10000000		
Corrected Total	24	2.64000000			

R-Square	Coeff Var	Root MSE	MORT Mean
0.242424	263.5231	0.316228	0.120000

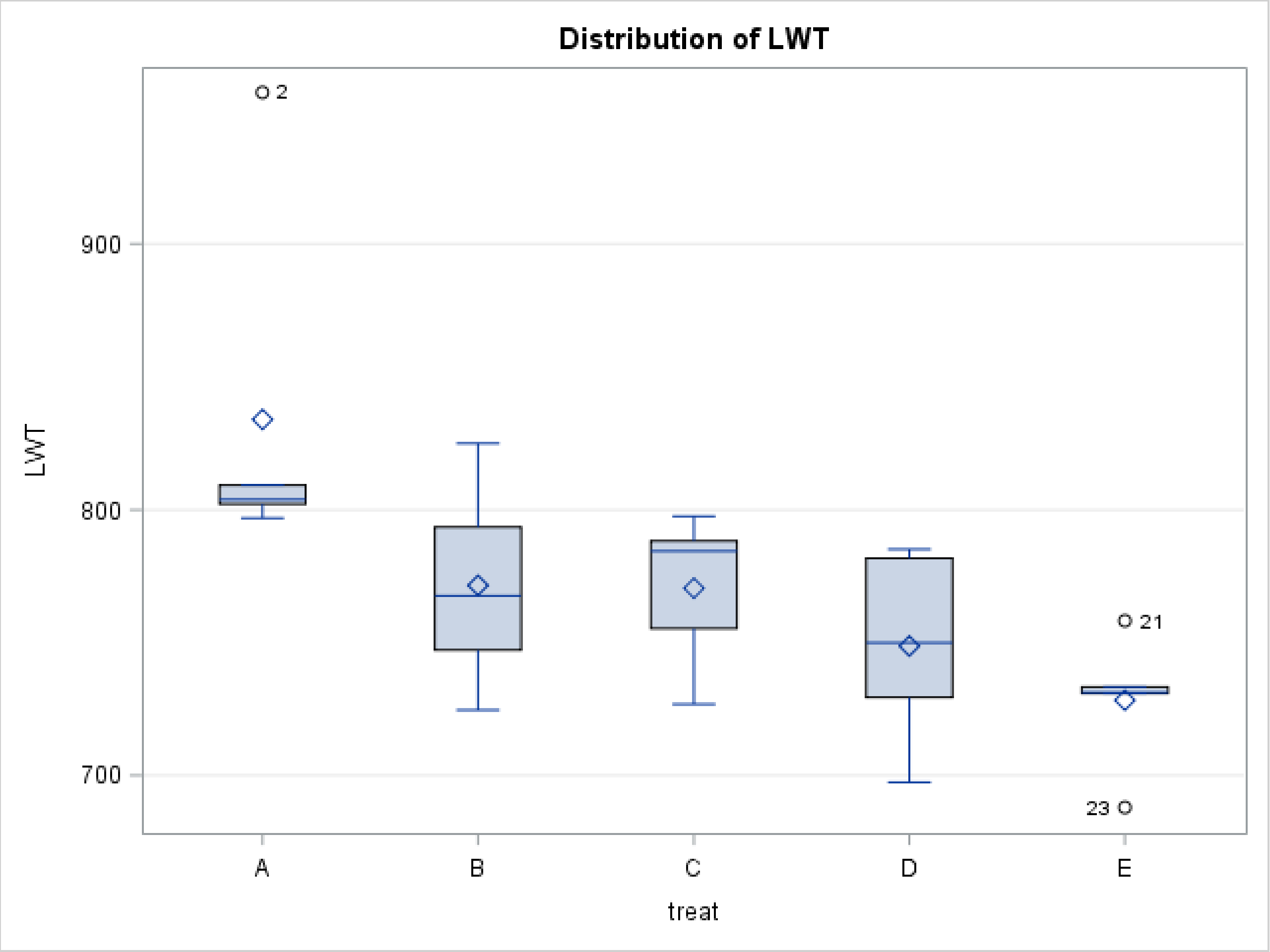
Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	0.64000000	0.16000000	1.60	0.2132

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	0.64000000	0.16000000	1.60	0.2132



The SAS System

The GLM Procedure



The GLM Procedure

t Tests (LSD) for LWT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	1827.145
Critical Value of t	2.08596
Least Significant Difference	56.393

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	833.87	5	A
B	771.56	5	B
B			
B	770.49	5	C
B			
B	748.72	5	D
B			
B	728.30	5	E

The GLM Procedure

Duncan's Multiple Range Test for LWT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	1827.145

Number of Means	**2**	**3**	**4**	**5**
Critical Range	56.39	59.19	60.97	62.22

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treat
A	833.87	5	A
B	771.56	5	B
B			
B	770.49	5	C
B			
B	748.72	5	D
B			
B	728.30	5	E

The GLM Procedure

Tukey's Studentized Range (HSD) Test for LWT

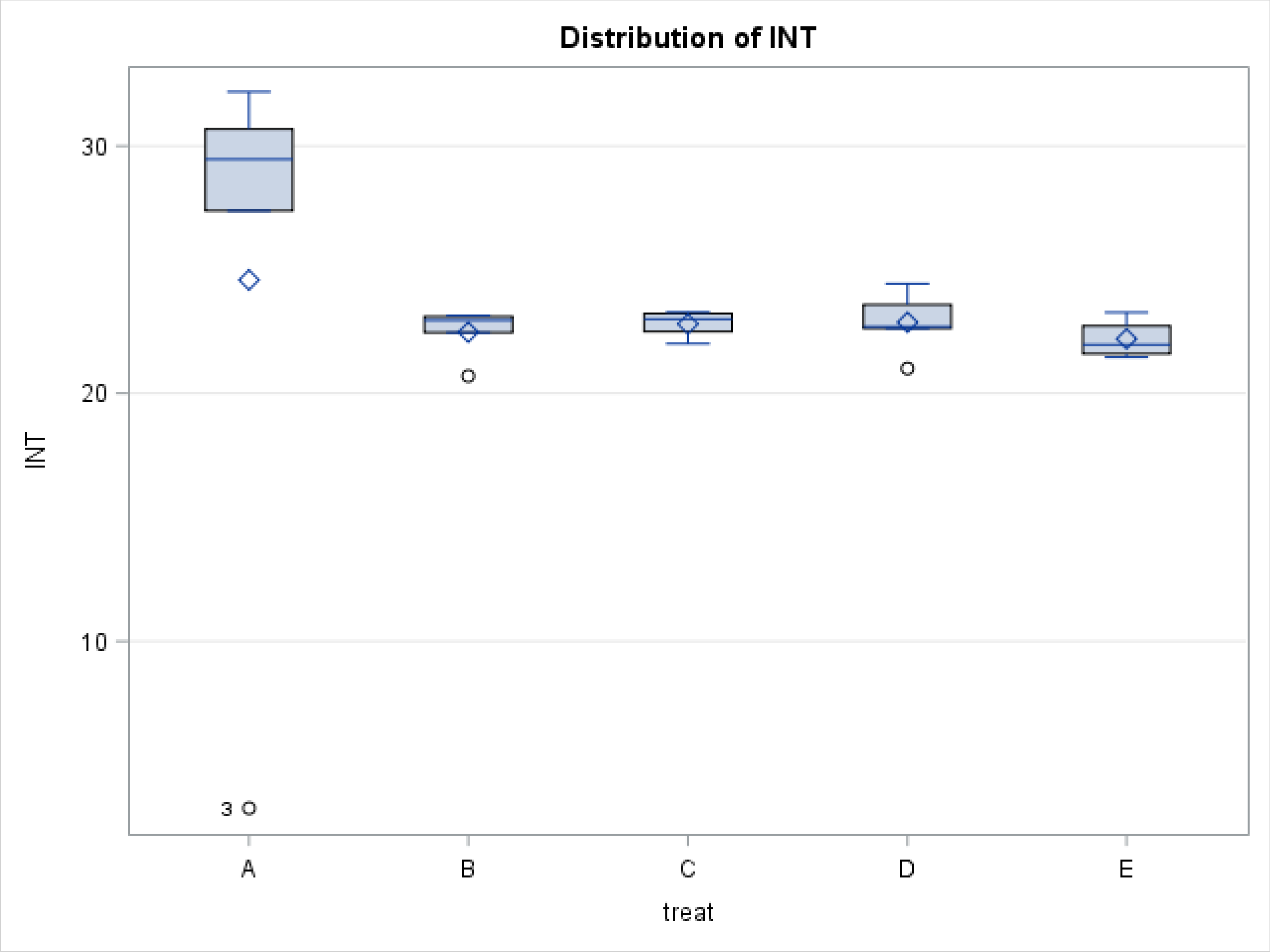
Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	1827.145
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	80.897

Means with the same letter are not significantly different.

Tukey Grouping		**Mean**	**N**	**treat**
	A	833.87	5	A
	A			
B	A	771.56	5	B
B	A			
B	A	770.49	5	C
B				
B		748.72	5	D
B				
B		728.30	5	E

The GLM Procedure



The GLM Procedure

t Tests (LSD) for INT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	29.81213
Critical Value of t	2.08596
Least Significant Difference	7.2033

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	24.592	5	A
A			
A	22.868	5	D
A			
A	22.800	5	C
A			
A	22.468	5	B
A			
A	22.198	5	E

The GLM Procedure

Duncan's Multiple Range Test for INT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	29.81213

Number of Means	**2**	**3**	**4**	**5**
Critical Range	7.203	7.561	7.788	7.947

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treat
A	24.592	5	A
A			
A	22.868	5	D
A			
A	22.800	5	C
A			
A	22.468	5	B
A			
A	22.198	5	E

The GLM Procedure

Tukey's Studentized Range (HSD) Test for INT

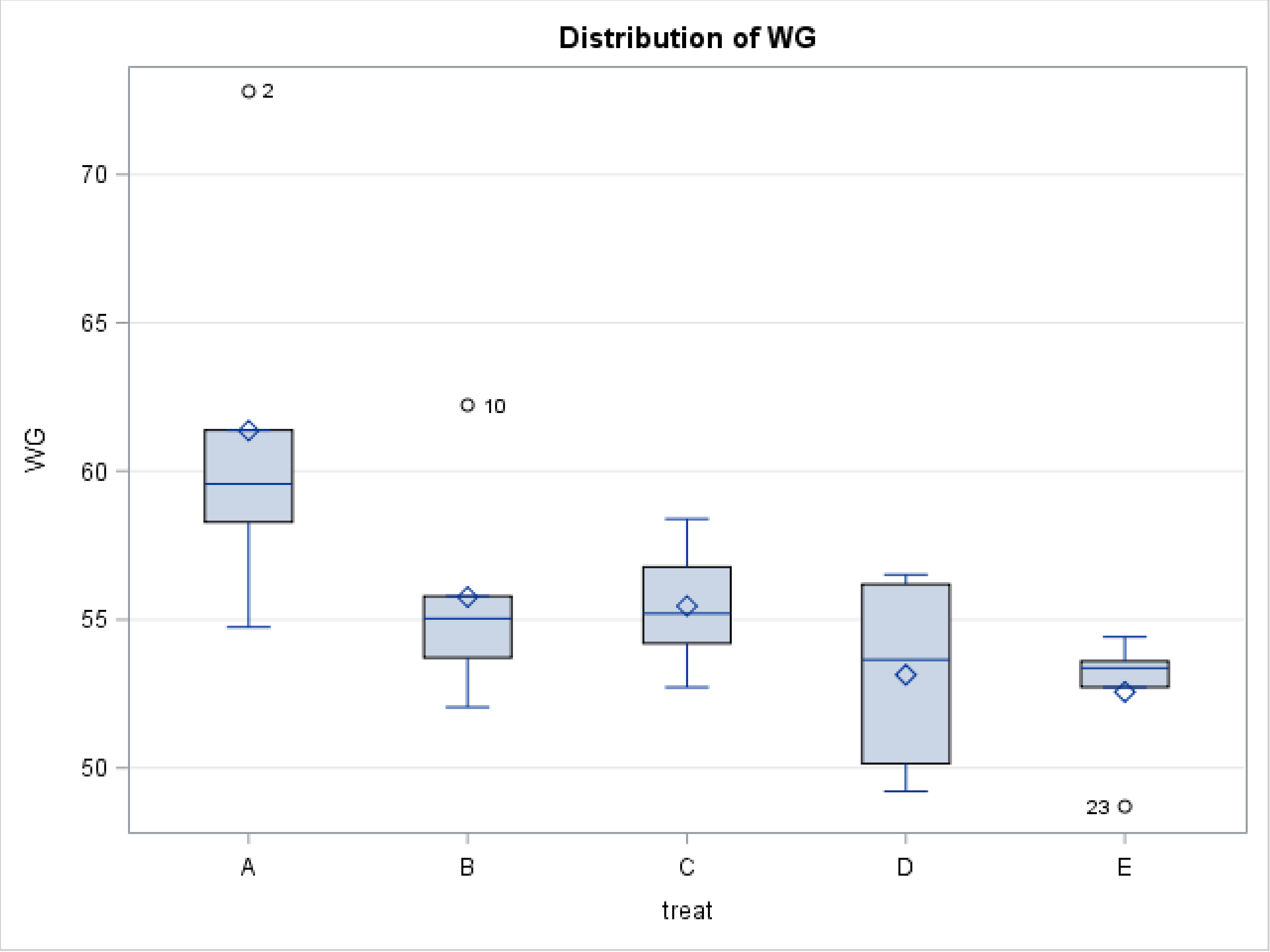
Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	29.81213
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	10.333

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	24.592	5	A
A			
A	22.868	5	D
A			
A	22.800	5	C
A			
A	22.468	5	B
A			
A	22.198	5	E

The GLM Procedure



The SAS System

The GLM Procedure

t Tests (LSD) for WG

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	16.6187
Critical Value of t	2.08596
Least Significant Difference	5.3782

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	61.360	5	A
B	55.764	5	B
B			
B	55.462	5	C
B			
B	53.144	5	D
B			
B	52.562	5	E

The GLM Procedure

Duncan's Multiple Range Test for WG

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	16.6187

Number of Means	**2**	**3**	**4**	**5**
Critical Range	5.378	5.645	5.815	5.934

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treat
A	61.360	5	A
B	55.764	5	B
B			
B	55.462	5	C
B			
B	53.144	5	D
B			
B	52.562	5	E

The GLM Procedure

Tukey's Studentized Range (HSD) Test for WG

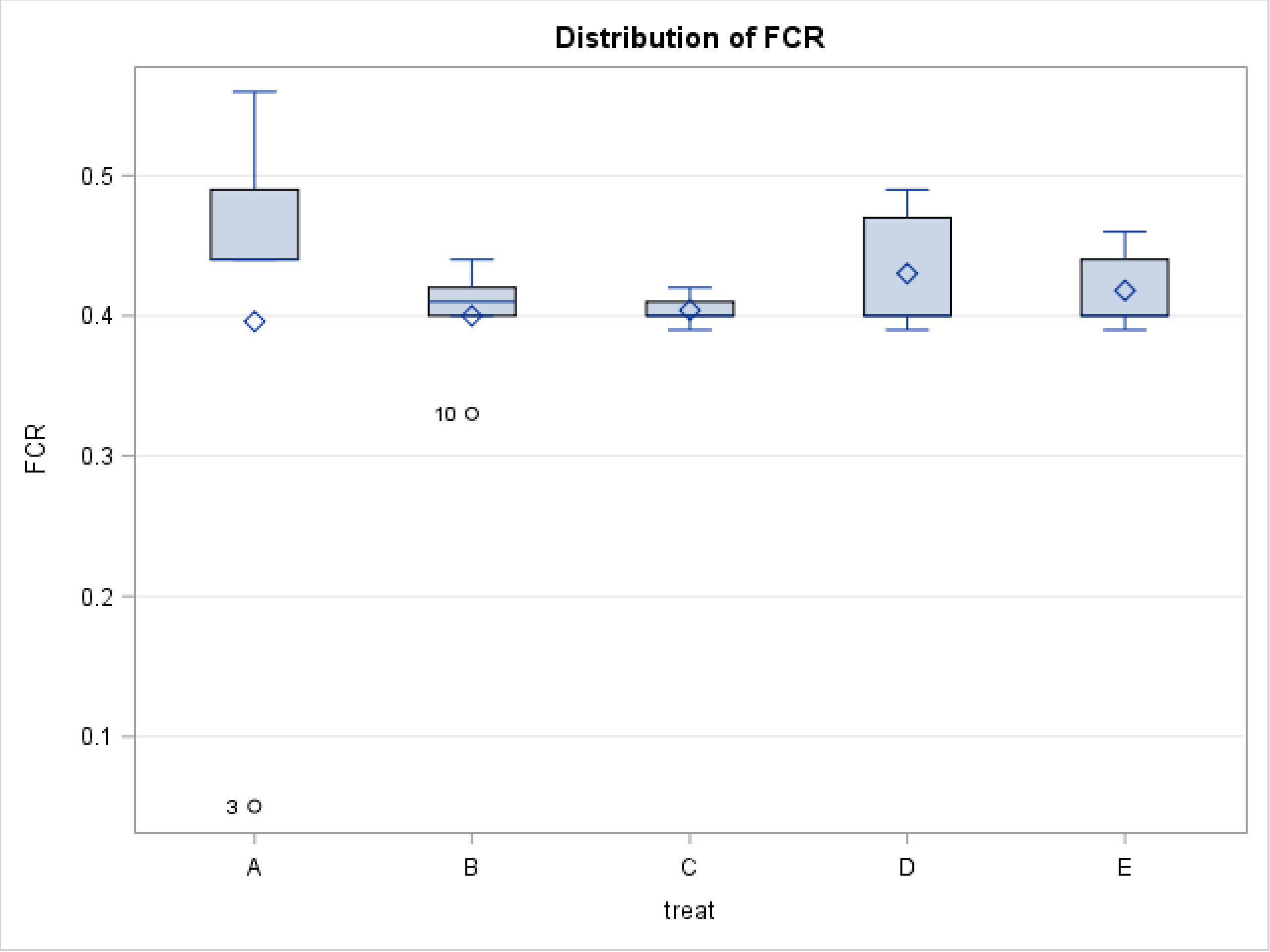
Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	16.6187
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	7.7152

Means with the same letter are not significantly different.

Tukey Grouping		**Mean**	**N**	**treat**
	A	61.360	5	A
	A			
B	A	55.764	5	B
B	A			
B	A	55.462	5	C
B				
B		53.144	5	D
B				
B		52.562	5	E

The GLM Procedure



The SAS System

The GLM Procedure

t Tests (LSD) for FCR

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.008956
Critical Value of t	2.08596
Least Significant Difference	0.1249

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	0.43000	5	D
A			
A	0.41800	5	E
A			
A	0.40400	5	C
A			
A	0.40000	5	B
A			
A	0.39600	5	A

The GLM Procedure

Duncan's Multiple Range Test for FCR

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.008956

Number of Means	**2**	**3**	**4**	**5**
Critical Range	.1248	.1311	.1350	.1377

Means with the same letter are not significantly different.

Duncan Grouping	**Mean**	**N**	**treat**
A	0.43000	5	D
A			
A	0.41800	5	E
A			
A	0.40400	5	C
A			
A	0.40000	5	B
A			
A	0.39600	5	A

The GLM Procedure

Tukey's Studentized Range (HSD) Test for FCR

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

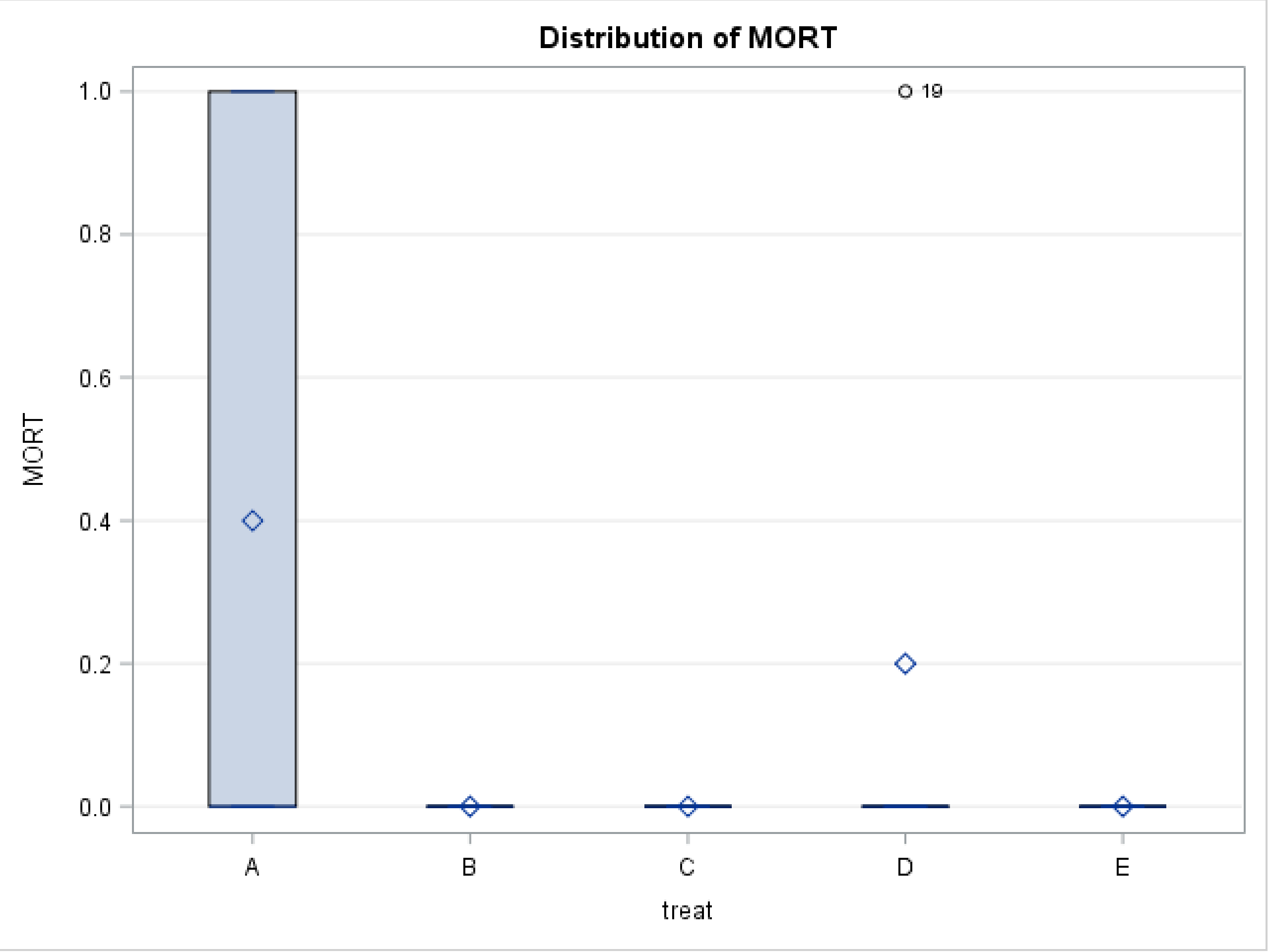
Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.008956
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.1791

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	0.43000	5	D
A			
A	0.41800	5	E
A			
A	0.40400	5	C
A			
A	0.40000	5	B
A			
A	0.39600	5	A

The SAS System

The GLM Procedure



The SAS System

The GLM Procedure

t Tests (LSD) for MORT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.1
Critical Value of t	2.08596
Least Significant Difference	0.4172

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	0.4000	5	A
A			
A	0.2000	5	D
A			
A	0.0000	5	C
A			
A	0.0000	5	B
A			
A	0.0000	5	E

The GLM Procedure

Duncan's Multiple Range Test for MORT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.1

Number of Means	**2**	**3**	**4**	**5**
Critical Range	.4172	.4379	.4511	.4603

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treat
A	0.4000	5	A
A			
A	0.2000	5	D
A			
A	0.0000	5	C
A			
A	0.0000	5	B
A			
A	0.0000	5	E

The GLM Procedure

Tukey's Studentized Range (HSD) Test for MORT

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.1
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.5985

Means with the same letter are not significantly different.

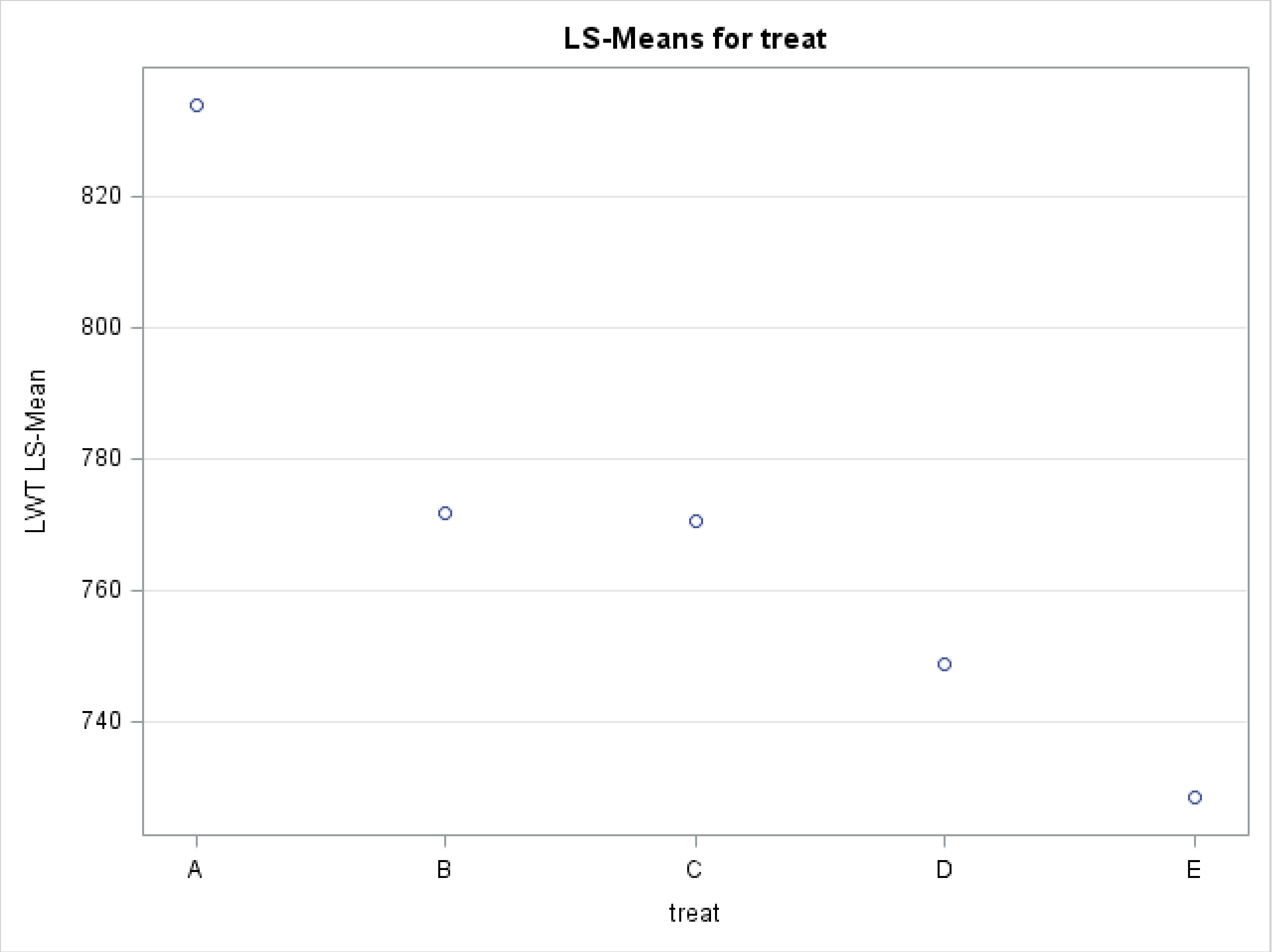
Tukey Grouping	Mean	N	treat
A	0.4000	5	A
A			
A	0.2000	5	D
A			
A	0.0000	5	C
A			
A	0.0000	5	B
A			
A	0.0000	5	E

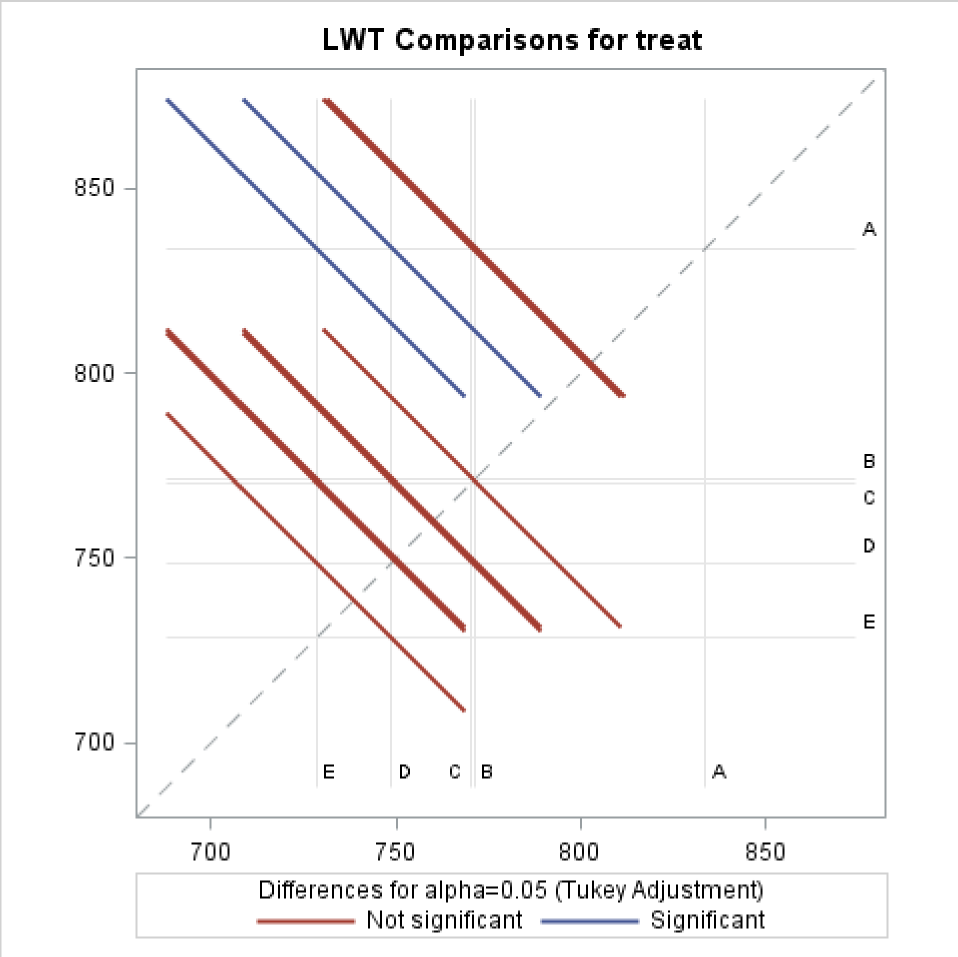
The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

treat	LWT LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	833.872000	19.116196	<.0001	1
B	771.560000	19.116196	<.0001	2
C	770.490000	19.116196	<.0001	3
D	748.720000	19.116196	<.0001	4
E	728.300000	19.116196	<.0001	5

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: LWT

i/j	1	2	3	4	5
1		0.1844	0.1722	0.0361	0.0070
2	0.1844		1.0000	0.9132	0.5141
3	0.1722	1.0000		0.9259	0.5377
4	0.0361	0.9132	0.9259		0.9403
5	0.0070	0.5141	0.5377	0.9403	


LS-Means for treat
820
800
780
760
740
LWT LS-Mean
A
B
C
D
E
treat



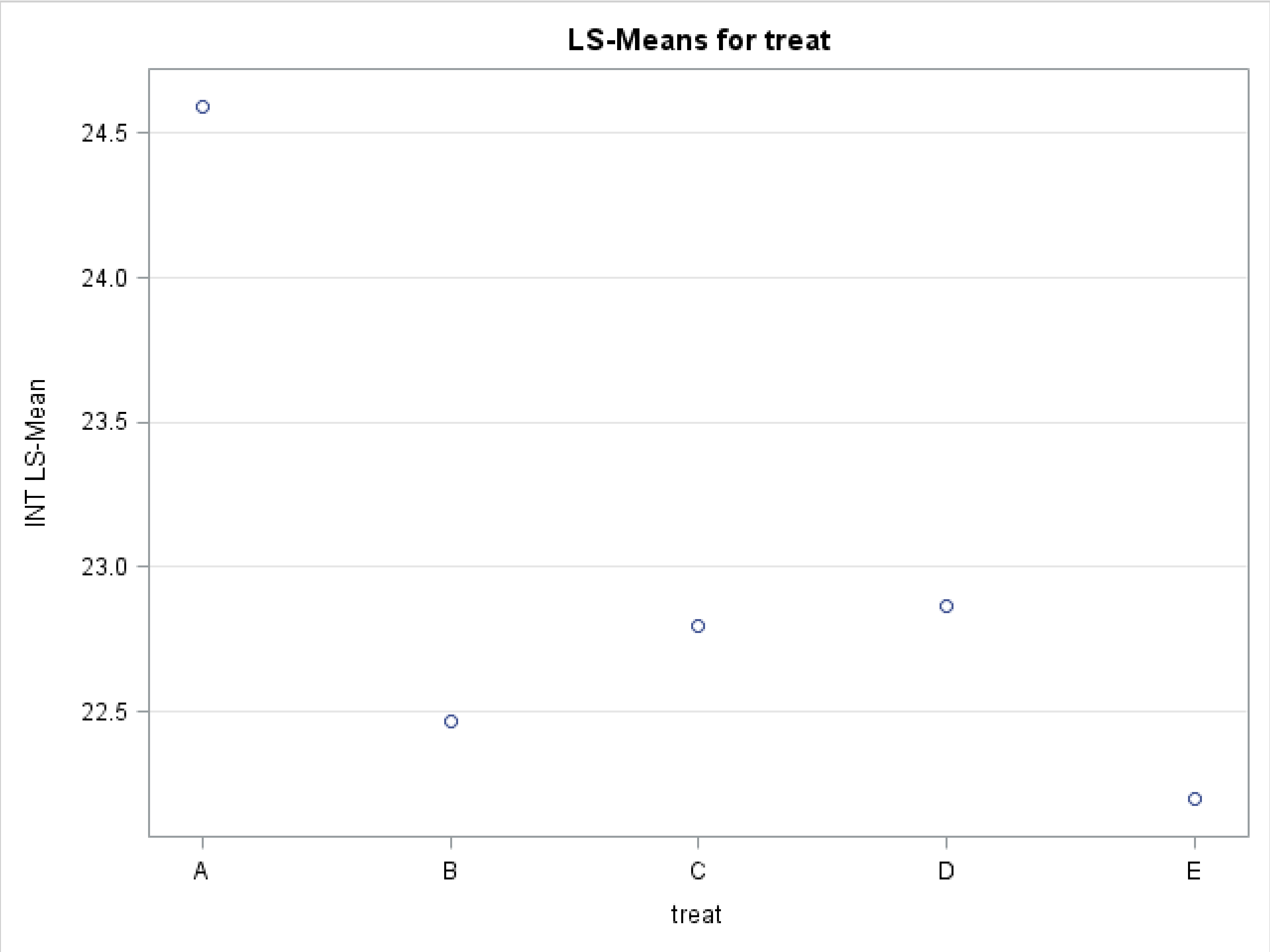
treat	INT LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	24.5920000	2.4418078	<.0001	1
B	22.4680000	2.4418078	<.0001	2
C	22.8000000	2.4418078	<.0001	3
D	22.8680000	2.4418078	<.0001	4
E	22.1980000	2.4418078	<.0001	5

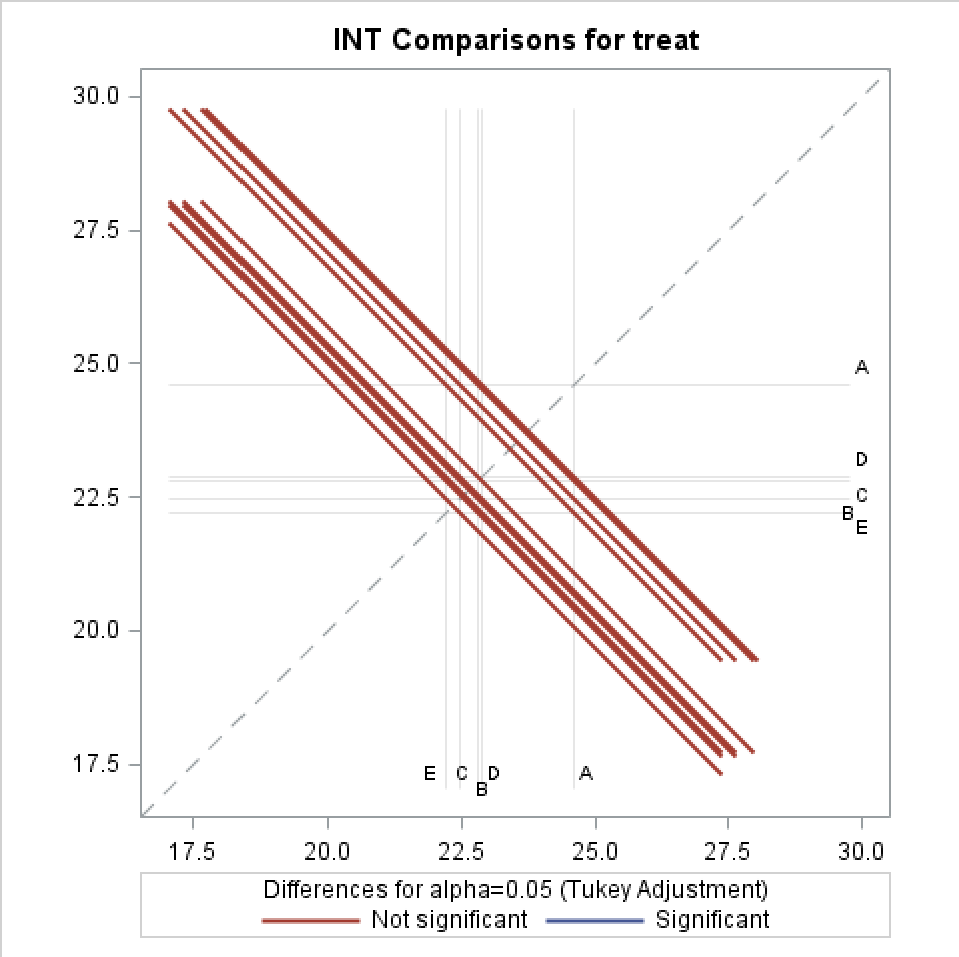
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: INT

i/j	1	2	3	4	5
1		0.9710	0.9844	0.9865	0.9556
2	0.9710		1.0000	1.0000	1.0000
3	0.9844	1.0000		1.0000	0.9998
4	0.9865	1.0000	1.0000		0.9997

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: INT

i/j	1	2	3	4	5
5	0.9556	1.0000	0.9998	0.9997	





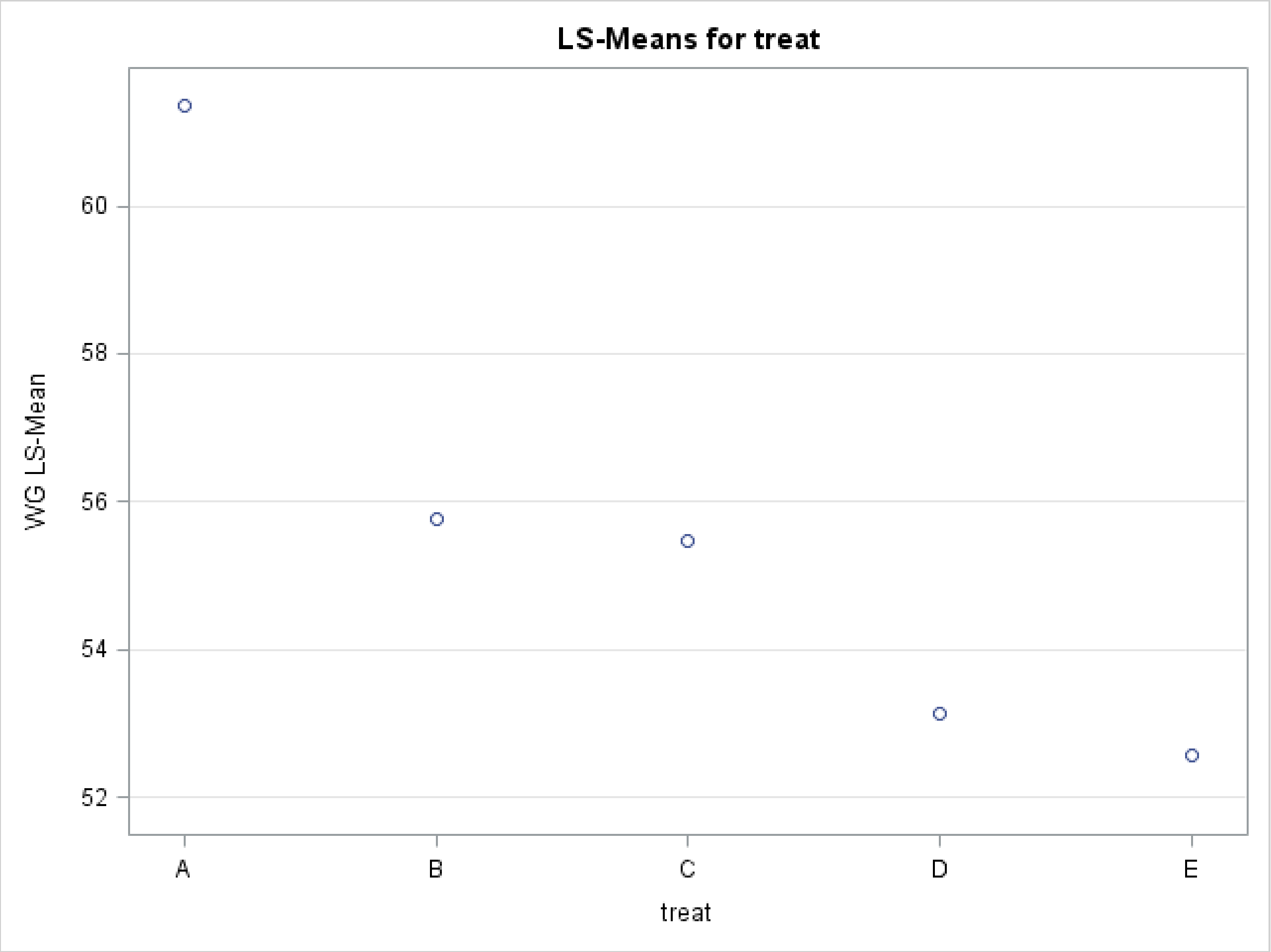
treat	WG LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	61.3600000	1.8231127	<.0001	1
B	55.7640000	1.8231127	<.0001	2
C	55.4620000	1.8231127	<.0001	3
D	53.1440000	1.8231127	<.0001	4
E	52.5620000	1.8231127	<.0001	5

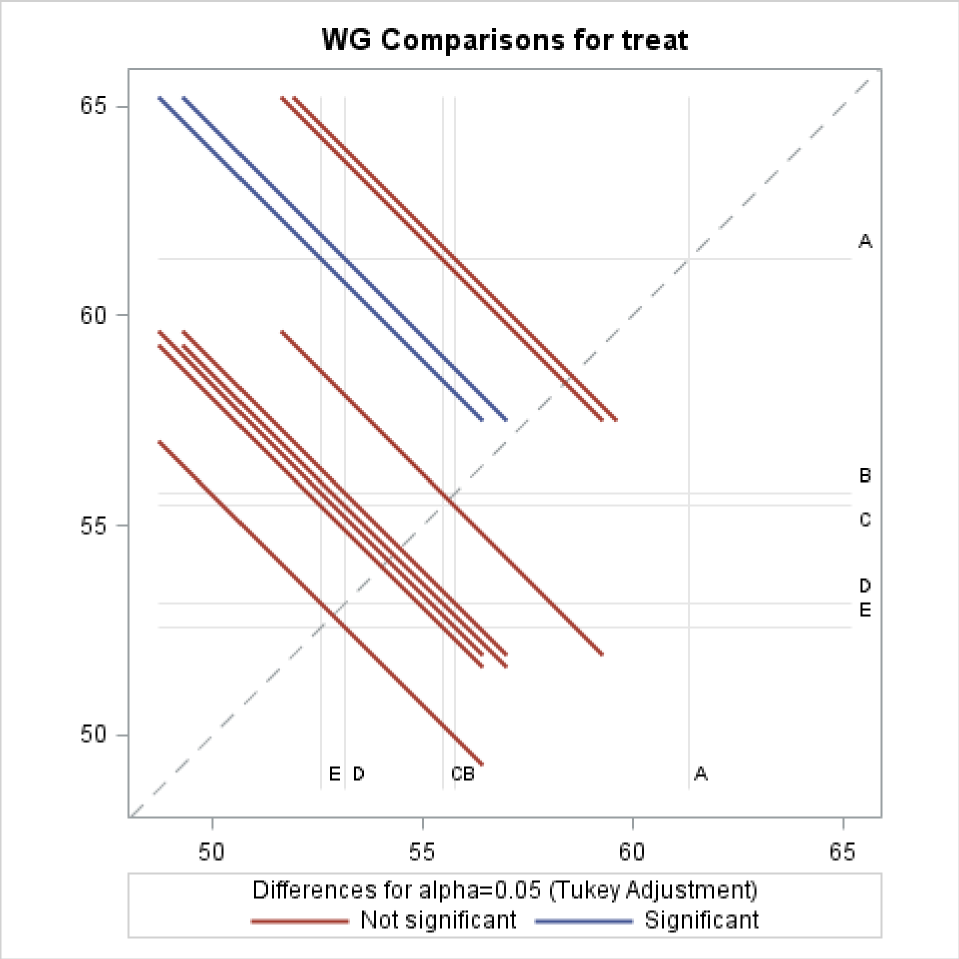
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: WG

i/j	1	2	3	4	5
1		0.2309	0.1899	0.0334	0.0206
2	0.2309		1.0000	0.8449	0.7278
3	0.1899	1.0000		0.8939	0.7918
4	0.0334	0.8449	0.8939		0.9994

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: WG

i/j	1	2	3	4	5
5	0.0206	0.7278	0.7918	0.9994	





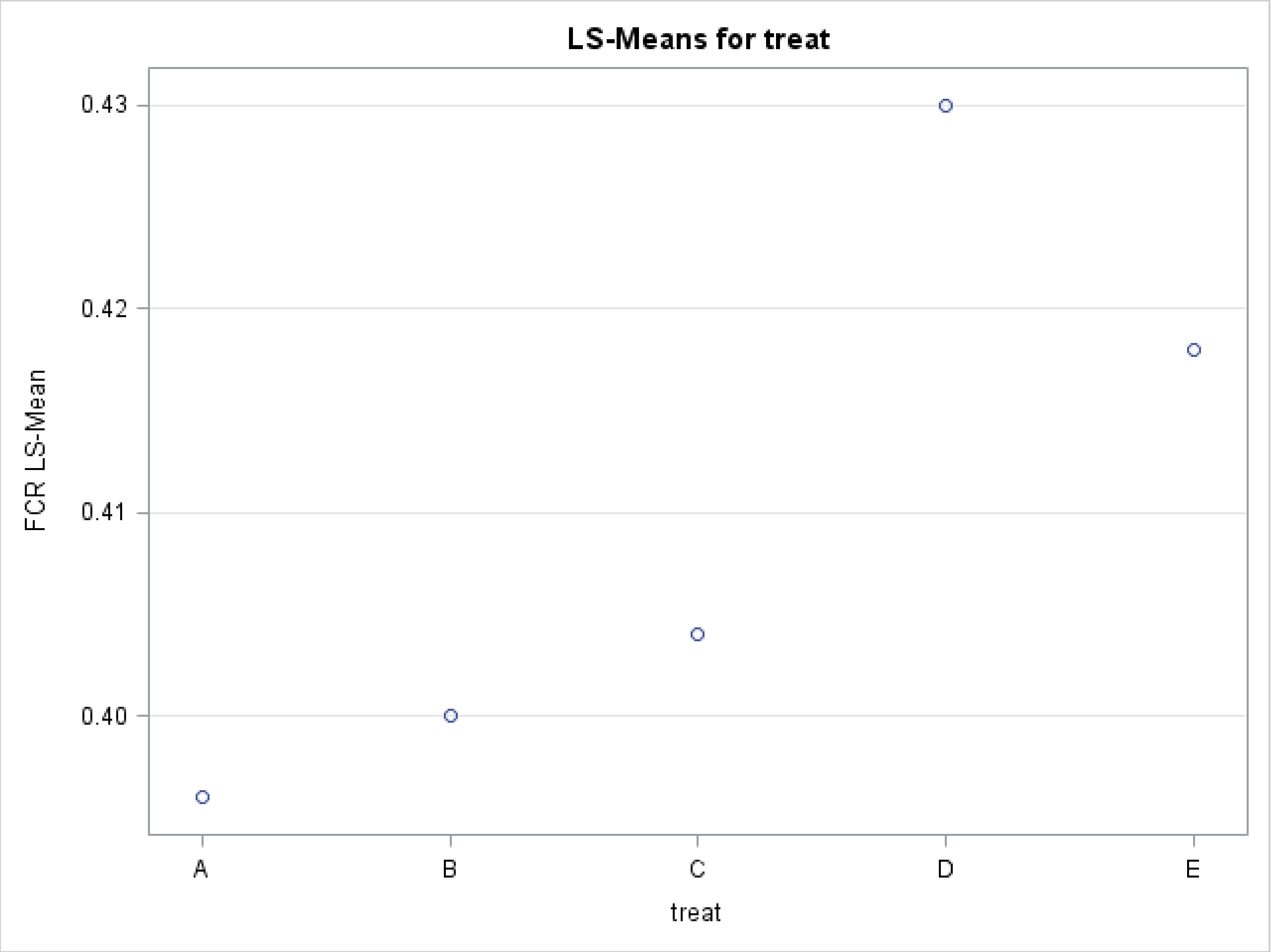
treat	FCR LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	0.39600000	0.04232257	<.0001	1
B	0.40000000	0.04232257	<.0001	2
C	0.40400000	0.04232257	<.0001	3
D	0.43000000	0.04232257	<.0001	4
E	0.41800000	0.04232257	<.0001	5

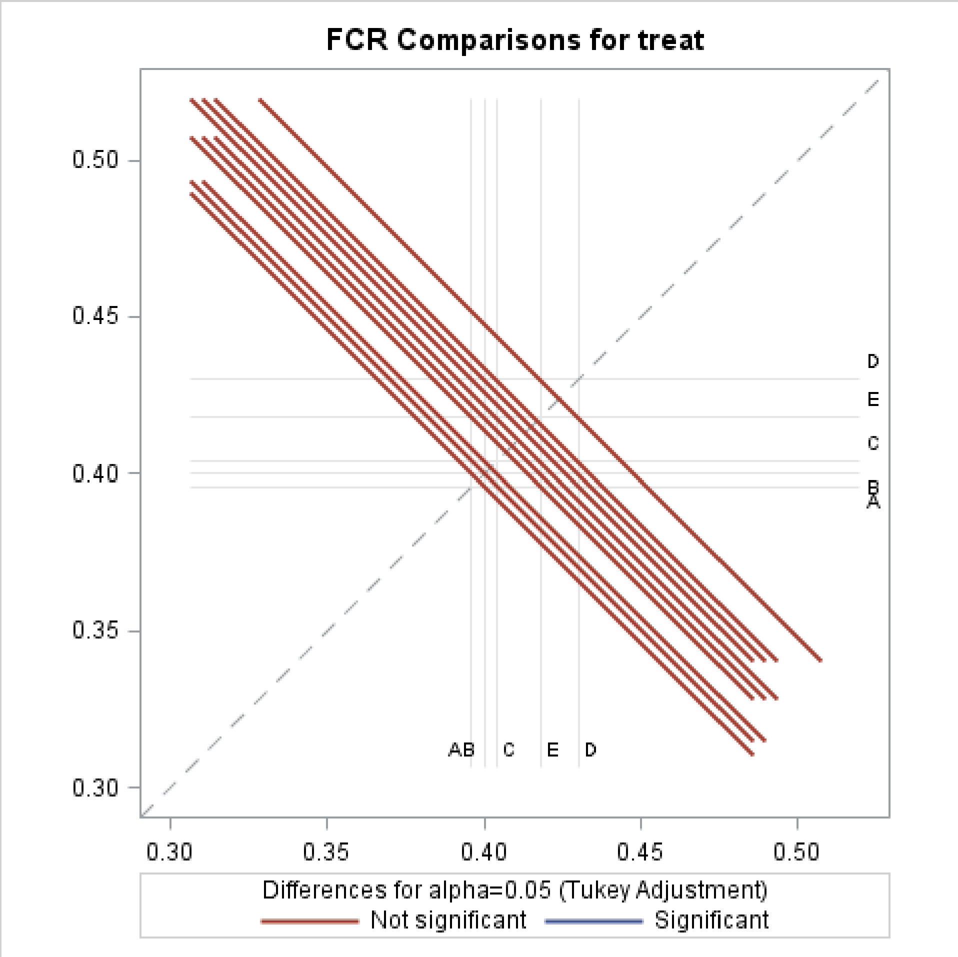
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: FCR

i/j	1	2	3	4	5
1		1.0000	0.9999	0.9783	0.9958
2	1.0000		1.0000	0.9863	0.9981
3	0.9999	1.0000		0.9920	0.9993
4	0.9783	0.9863	0.9920		0.9996

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: FCR

i/j	1	2	3	4	5
5	0.9958	0.9981	0.9993	0.9996	





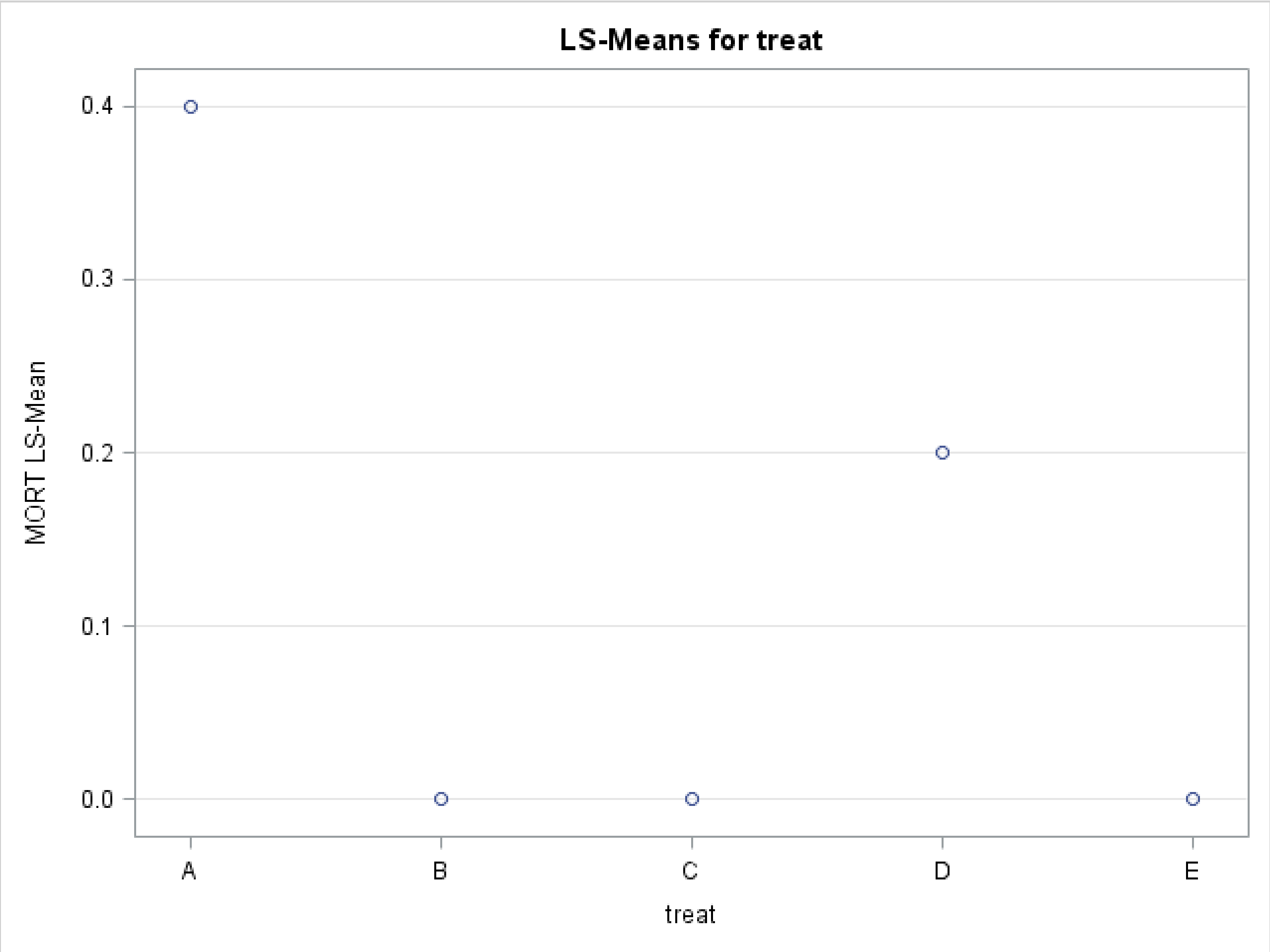
treat	MORT LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	0.40000000	0.14142136	0.0104	1
B	0.00000000	0.14142136	1.0000	2
C	0.00000000	0.14142136	1.0000	3
D	0.20000000	0.14142136	0.1727	4
E	0.00000000	0.14142136	1.0000	5

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: MORT

i/j	1	2	3	4	5
1		0.3016	0.3016	0.8523	0.3016
2	0.3016		1.0000	0.8523	1.0000
3	0.3016	1.0000		0.8523	1.0000
4	0.8523	0.8523	0.8523		0.8523

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: MORT

i/j	1	2	3	4	5
5	0.3016	1.0000	1.0000	0.8523	



The GLM Procedure

Class Level Information

Class	Levels	Values
treat	5	A B C D E

Number of Observations Read	25
Number of Observations Used	25

The SAS System

The GLM Procedure

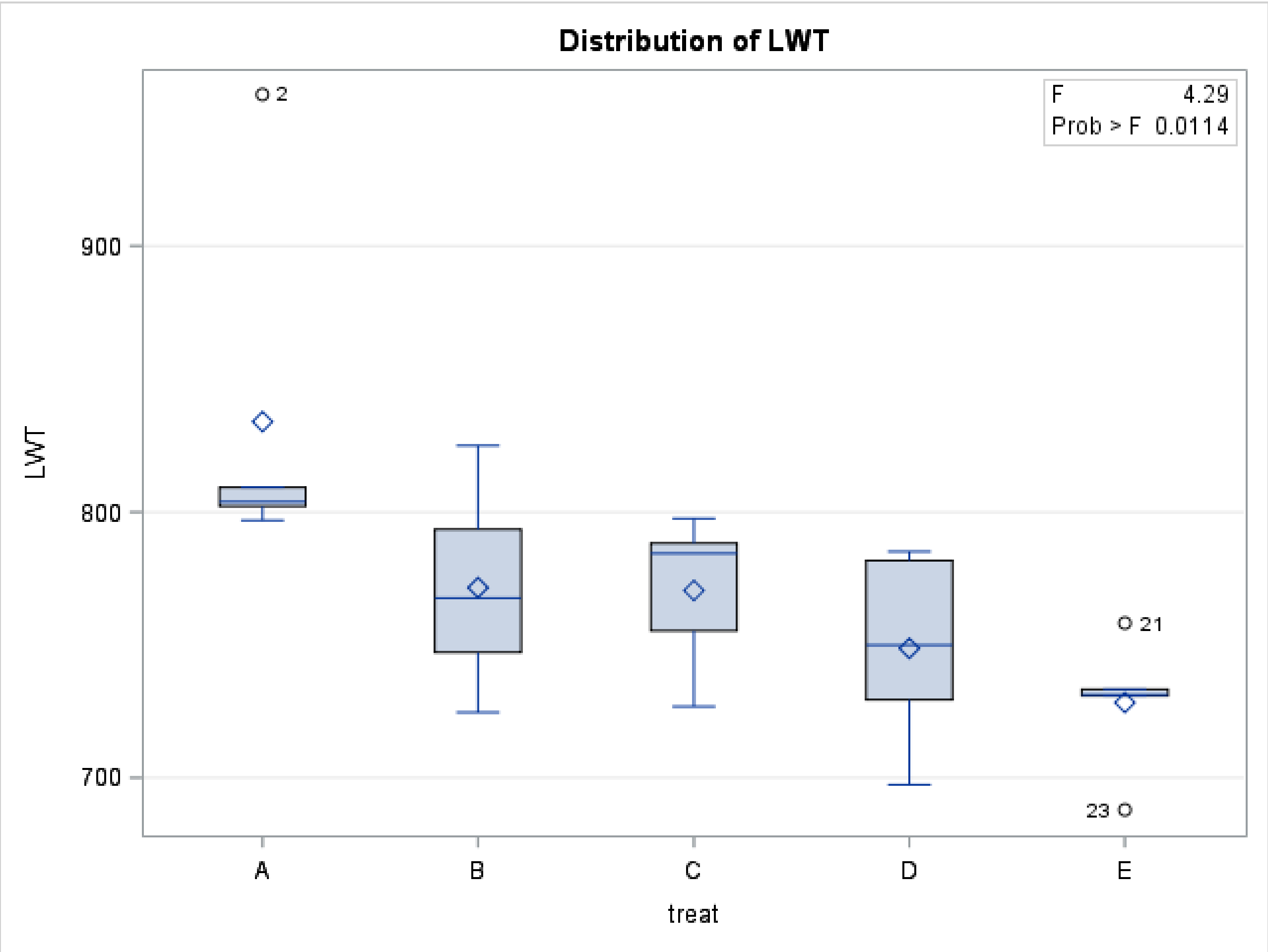
Dependent Variable: LWT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	31361.51706	7840.37926	4.29	0.0114
Error	20	36542.89668	1827.14483		
Corrected Total	24	67904.41374			

R-Square	Coeff Var	Root MSE	LWT Mean
0.461848	5.547075	42.74511	770.5884

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	31361.51706	7840.37926	4.29	0.0114

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	31361.51706	7840.37926	4.29	0.0114



The GLM Procedure

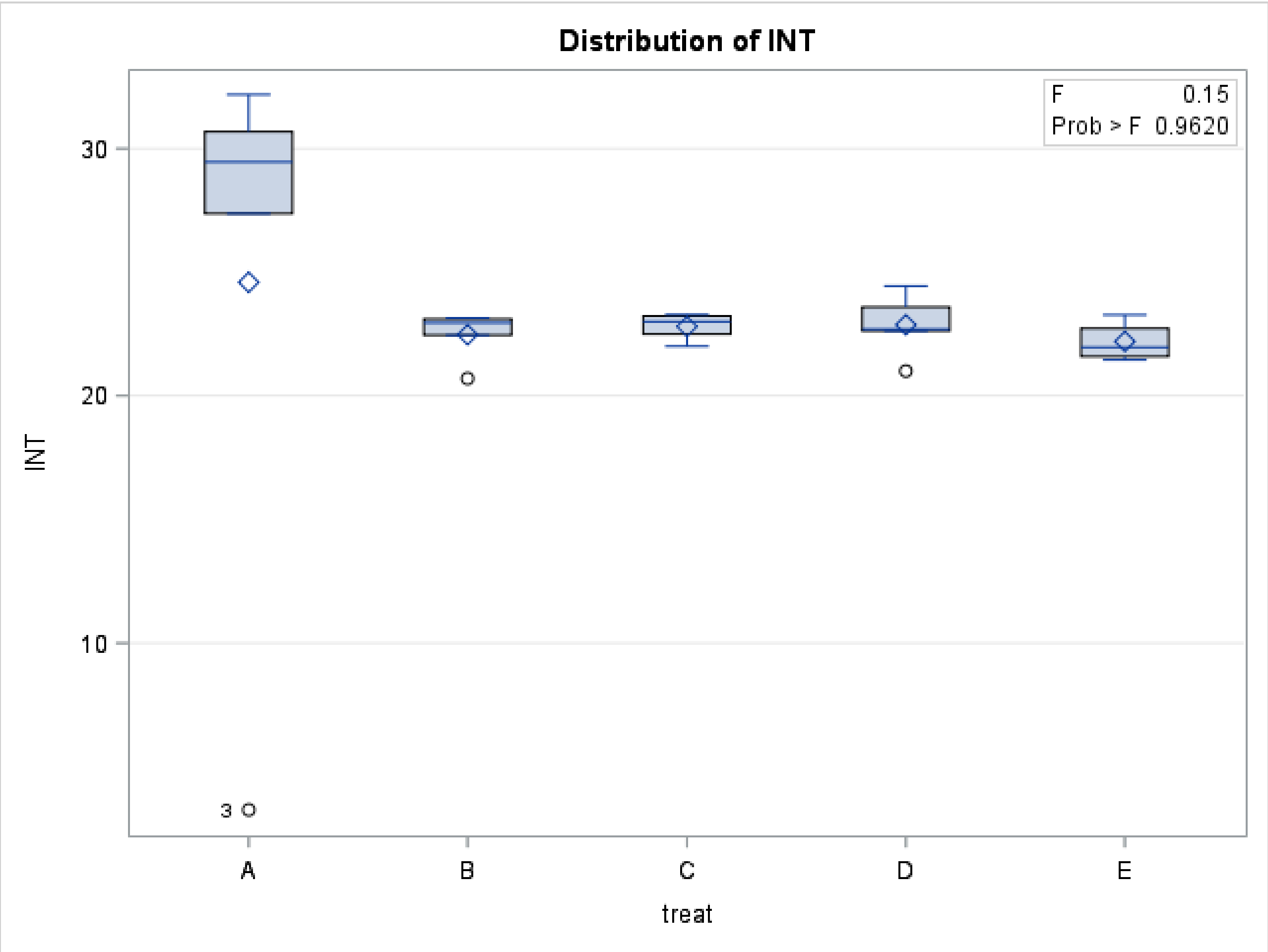
Dependent Variable: INT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	17.5851040	4.3962760	0.15	0.9620
Error	20	596.2425200	29.8121260		
Corrected Total	24	613.8276240			

R-Square	Coeff Var	Root MSE	INT Mean
0.028648	23.75463	5.460048	22.98520

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	17.58510400	4.39627600	0.15	0.9620

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	17.58510400	4.39627600	0.15	0.9620



The SAS System

The GLM Procedure

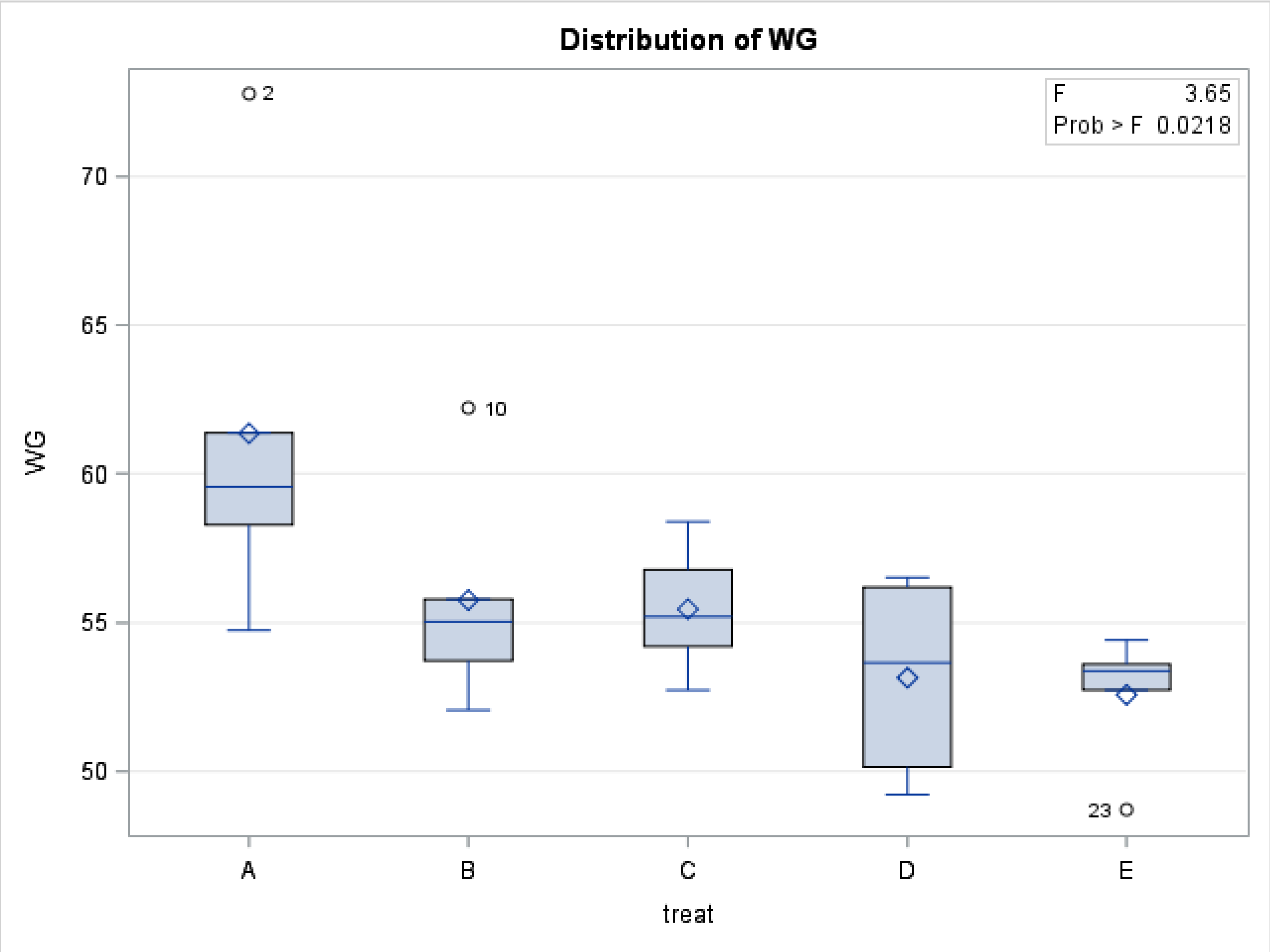
Dependent Variable: WG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	242.3393360	60.5848340	3.65	0.0218
Error	20	332.3740000	16.6187000		
Corrected Total	24	574.7133360			

R-Square	Coeff Var	Root MSE	WG Mean
0.421670	7.324328	4.076604	55.65840

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	242.3393360	60.5848340	3.65	0.0218

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	242.3393360	60.5848340	3.65	0.0218



The SAS System

The GLM Procedure

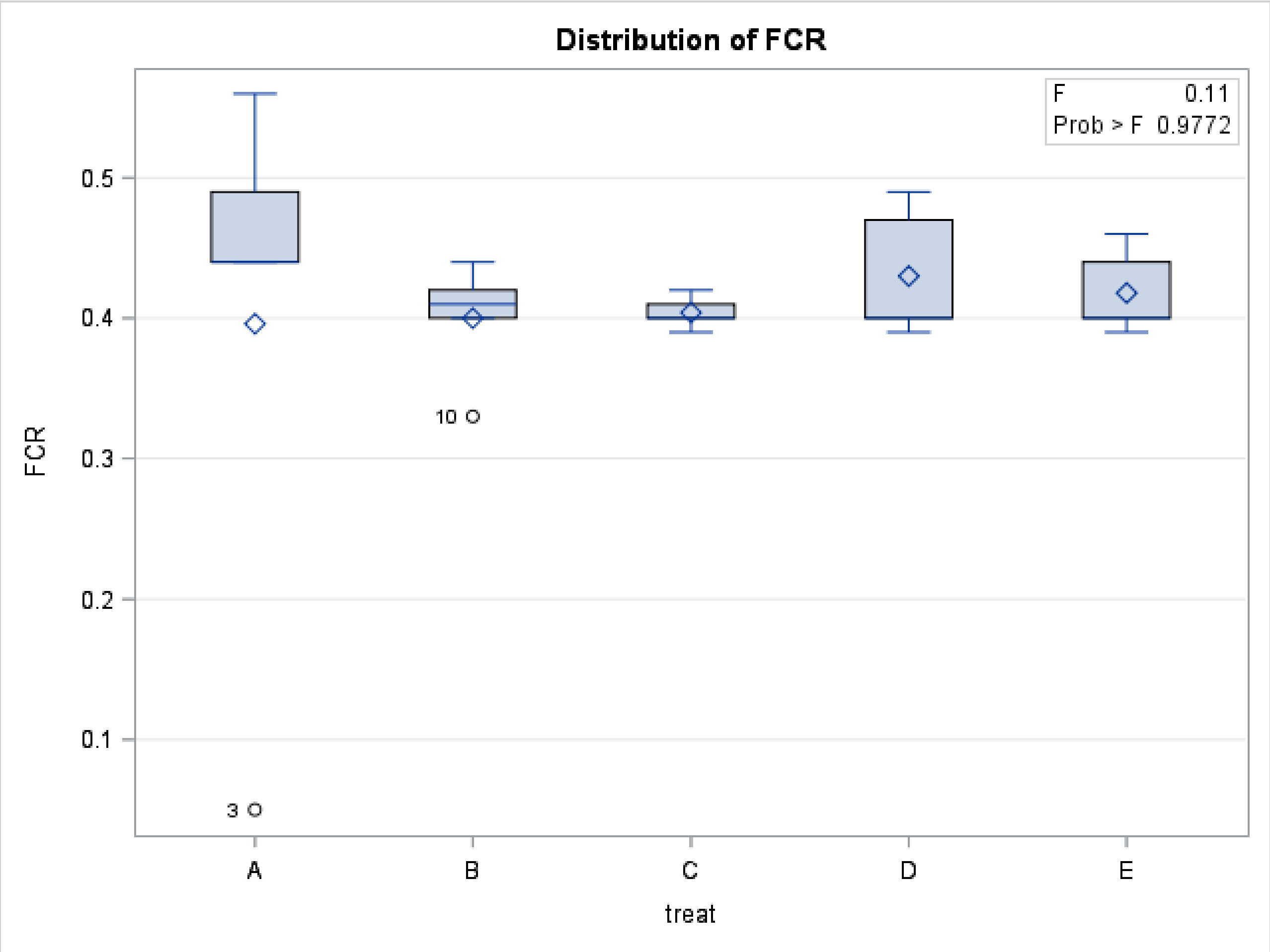
Dependent Variable: FCR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.00397600	0.00099400	0.11	0.9772
Error	20	0.17912000	0.00895600		
Corrected Total	24	0.18309600			

R-Square	Coeff Var	Root MSE	FCR Mean
0.021715	23.10453	0.094636	0.409600

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	0.00397600	0.00099400	0.11	0.9772

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	0.00397600	0.00099400	0.11	0.9772



The SAS System

The GLM Procedure

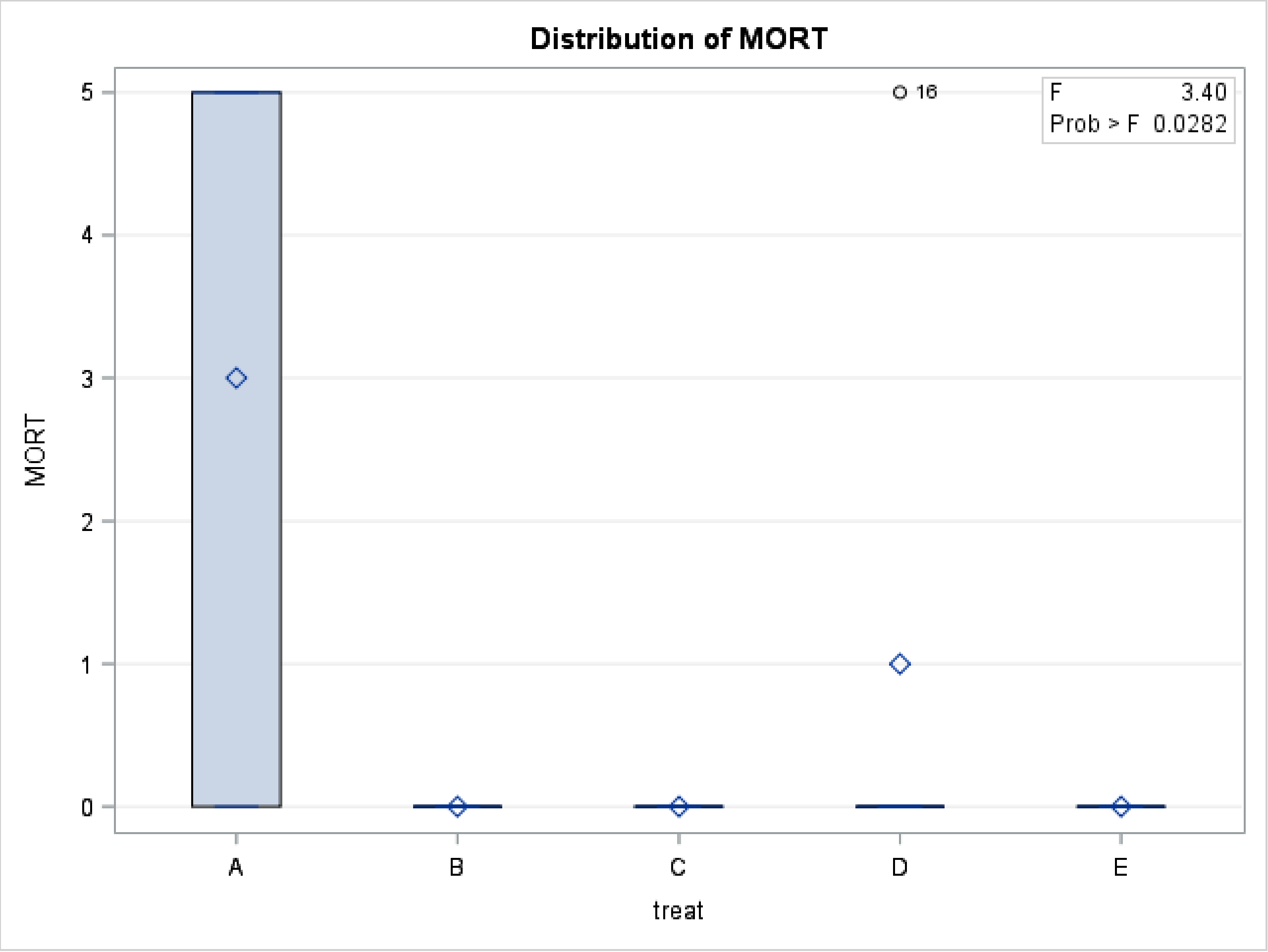
Dependent Variable: MORT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	34.00000000	8.50000000	3.40	0.0282
Error	20	50.00000000	2.50000000		
Corrected Total	24	84.00000000			

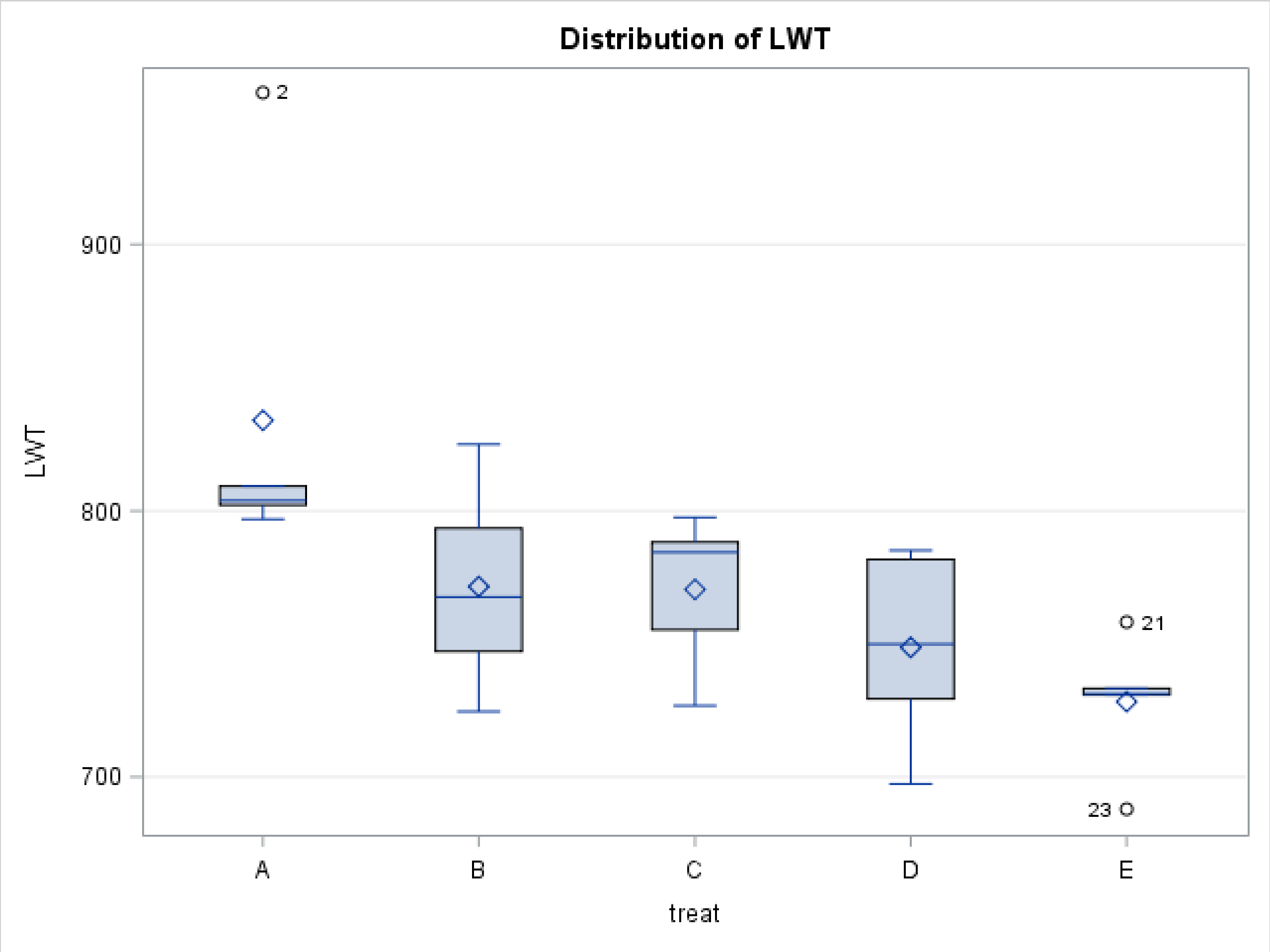
R-Square	Coeff Var	Root MSE	MORT Mean
0.404762	197.6424	1.581139	0.800000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	34.00000000	8.50000000	3.40	0.0282

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	34.00000000	8.50000000	3.40	0.0282



The GLM Procedure



The SAS System

The GLM Procedure

t Tests (LSD) for LWT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	1827.145
Critical Value of t	2.08596
Least Significant Difference	56.393

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	833.87	5	A
B	771.56	5	B
B			
B	770.49	5	C
B			
B	748.72	5	D
B			
B	728.30	5	E

The SAS System

The GLM Procedure

Duncan's Multiple Range Test for LWT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	1827.145

Number of Means	**2**	**3**	**4**	**5**
Critical Range	56.39	59.19	60.97	62.22

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treat
A	833.87	5	A
B	771.56	5	B
B			
B	770.49	5	C
B			
B	748.72	5	D
B			
B	728.30	5	E

The GLM Procedure

Tukey's Studentized Range (HSD) Test for LWT

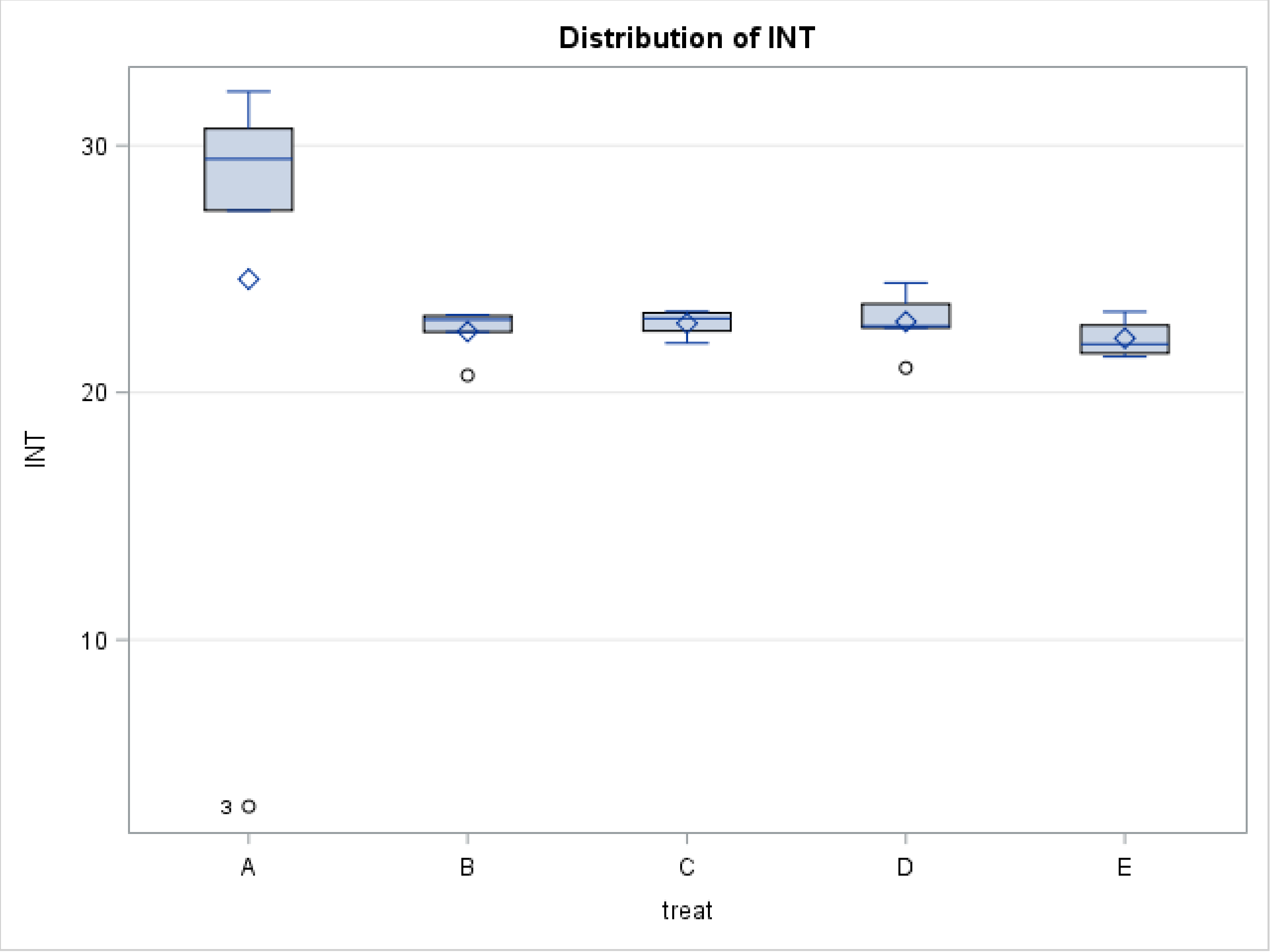
Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	1827.145
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	80.897

Means with the same letter are not significantly different.

Tukey Grouping		**Mean**	**N**	**treat**
	A	833.87	5	A
	A			
B	A	771.56	5	B
B	A			
B	A	770.49	5	C
B				
B		748.72	5	D
B				
B		728.30	5	E

The GLM Procedure



The SAS System

The GLM Procedure

t Tests (LSD) for INT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	29.81213
Critical Value of t	2.08596
Least Significant Difference	7.2033

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	24.592	5	A
A			
A	22.868	5	D
A			
A	22.800	5	C
A			
A	22.468	5	B
A			
A	22.198	5	E

The GLM Procedure

Duncan's Multiple Range Test for INT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	29.81213

Number of Means	**2**	**3**	**4**	**5**
Critical Range	7.203	7.561	7.788	7.947

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treat
A	24.592	5	A
A			
A	22.868	5	D
A			
A	22.800	5	C
A			
A	22.468	5	B
A			
A	22.198	5	E

The SAS System

The GLM Procedure

Tukey's Studentized Range (HSD) Test for INT

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

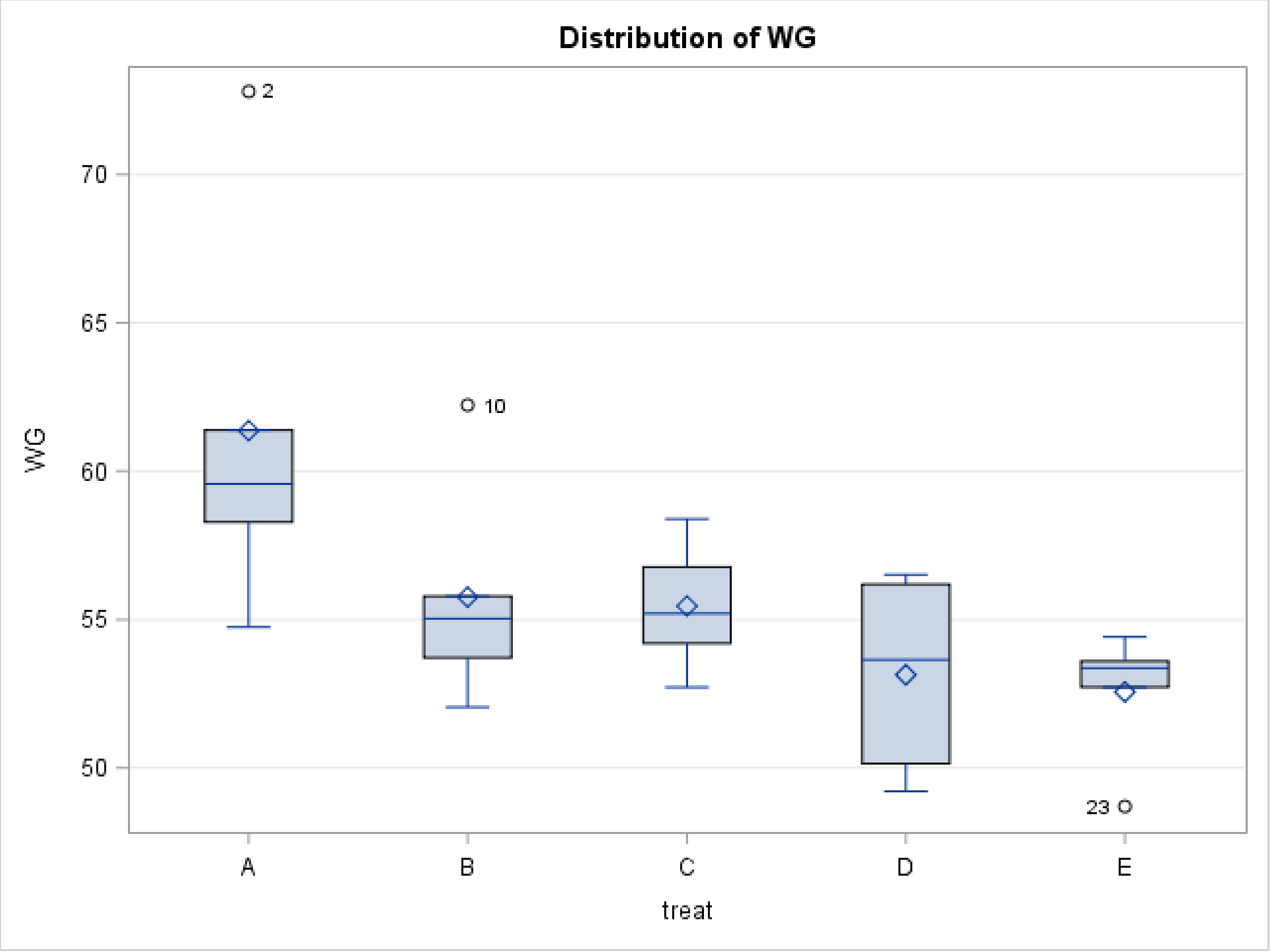
Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	29.81213
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	10.333

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	24.592	5	A
A			
A	22.868	5	D
A			
A	22.800	5	C
A			
A	22.468	5	B
A			
A	22.198	5	E

The SAS System

The GLM Procedure



The SAS System

The GLM Procedure

t Tests (LSD) for WG

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	16.6187
Critical Value of t	2.08596
Least Significant Difference	5.3782

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	61.360	5	A
B	55.764	5	B
B			
B	55.462	5	C
B			
B	53.144	5	D
B			
B	52.562	5	E

The GLM Procedure

Duncan's Multiple Range Test for WG

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	16.6187

Number of Means	**2**	**3**	**4**	**5**
Critical Range	5.378	5.645	5.815	5.934

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treat
A	61.360	5	A
B	55.764	5	B
B			
B	55.462	5	C
B			
B	53.144	5	D
B			
B	52.562	5	E

The GLM Procedure

Tukey's Studentized Range (HSD) Test for WG

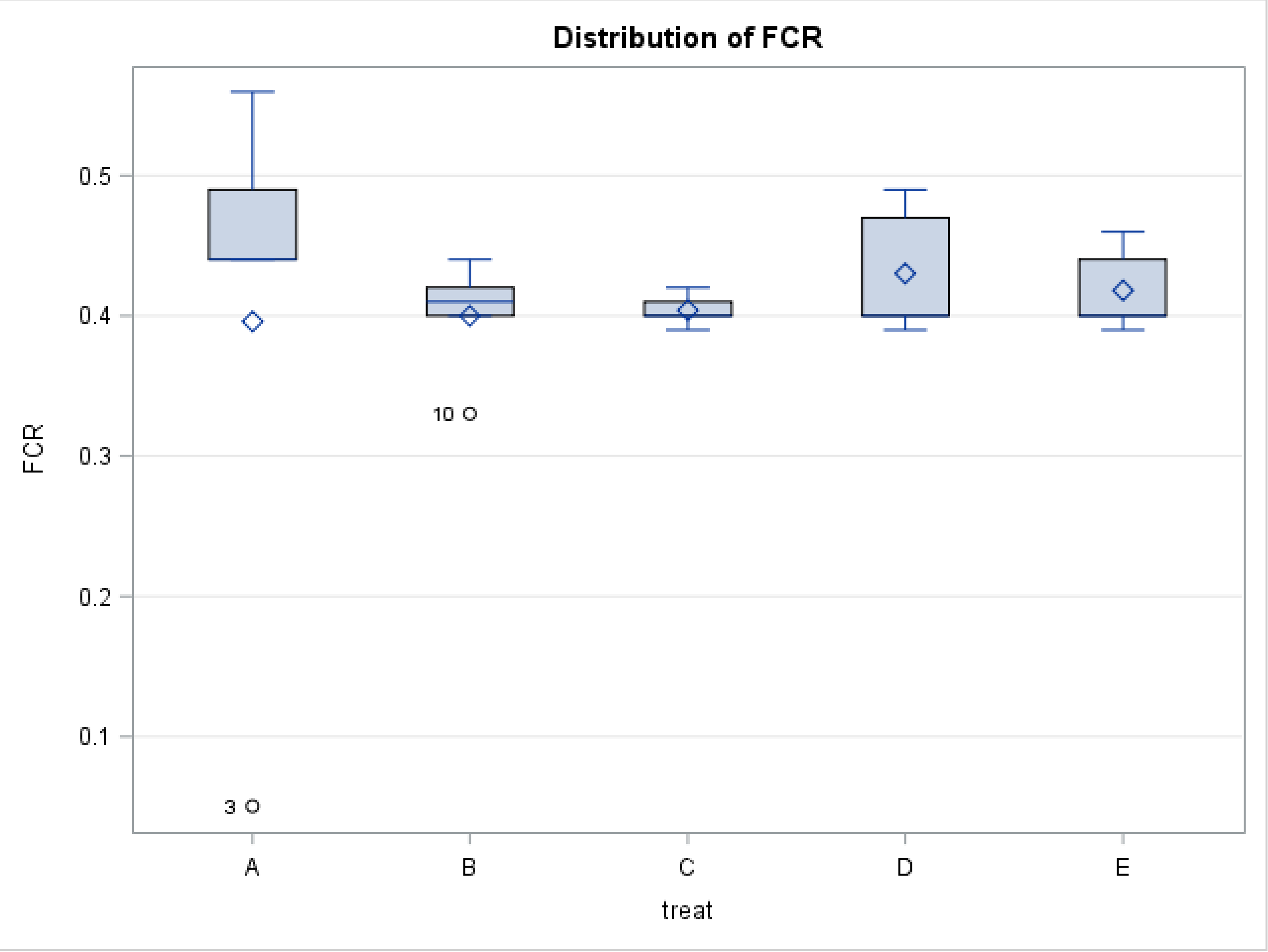
Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	16.6187
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	7.7152

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	treat
	A	61.360	5	A
	A			
B	A	55.764	5	B
B	A			
B	A	55.462	5	C
B				
B		53.144	5	D
B				
B		52.562	5	E

The GLM Procedure



The GLM Procedure

t Tests (LSD) for FCR

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.008956
Critical Value of t	2.08596
Least Significant Difference	0.1249

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	0.43000	5	D
A			
A	0.41800	5	E
A			
A	0.40400	5	C
A			
A	0.40000	5	B
A			
A	0.39600	5	A

The GLM Procedure

Duncan's Multiple Range Test for FCR

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.008956

Number of Means	**2**	**3**	**4**	**5**
Critical Range	.1248	.1311	.1350	.1377

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treat
A	0.43000	5	D
A			
A	0.41800	5	E
A			
A	0.40400	5	C
A			
A	0.40000	5	B
A			
A	0.39600	5	A

The GLM Procedure

Tukey's Studentized Range (HSD) Test for FCR

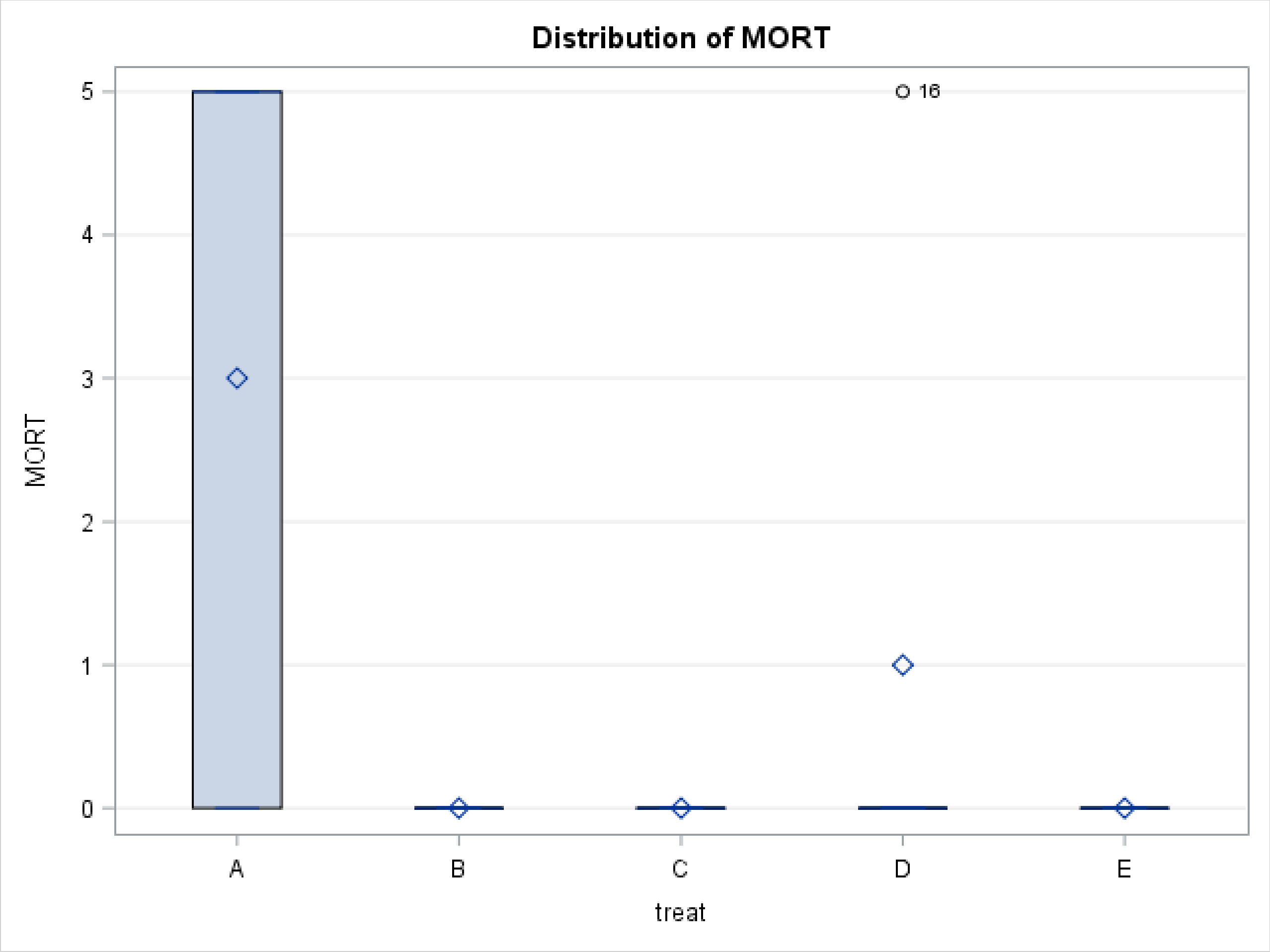
Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.008956
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.1791

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	0.43000	5	D
A			
A	0.41800	5	E
A			
A	0.40400	5	C
A			
A	0.40000	5	B
A			
A	0.39600	5	A

The GLM Procedure



The GLM Procedure

t Tests (LSD) for MORT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	2.5
Critical Value of t	2.08596
Least Significant Difference	2.086

Means with the same letter are not significantly different.

t Grouping		Mean	N	treat
	A	3.000	5	A
	A			
B	A	1.000	5	D
B				
B		0.000	5	C
B				
B		0.000	5	B
B				
B		0.000	5	E

The GLM Procedure

Duncan's Multiple Range Test for MORT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	2.5

Number of Means	**2**	**3**	**4**	**5**
Critical Range	2.086	2.190	2.255	2.301

Means with the same letter are not significantly different.

Duncan Grouping		**Mean**	**N**	**treat**
	A	3.000	5	A
	A			
B	A	1.000	5	D
B				
B		0.000	5	C
B				
B		0.000	5	B
B				
B		0.000	5	E

The GLM Procedure

Tukey's Studentized Range (HSD) Test for MORT

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	2.5
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	2.9924

Means with the same letter are not significantly different.

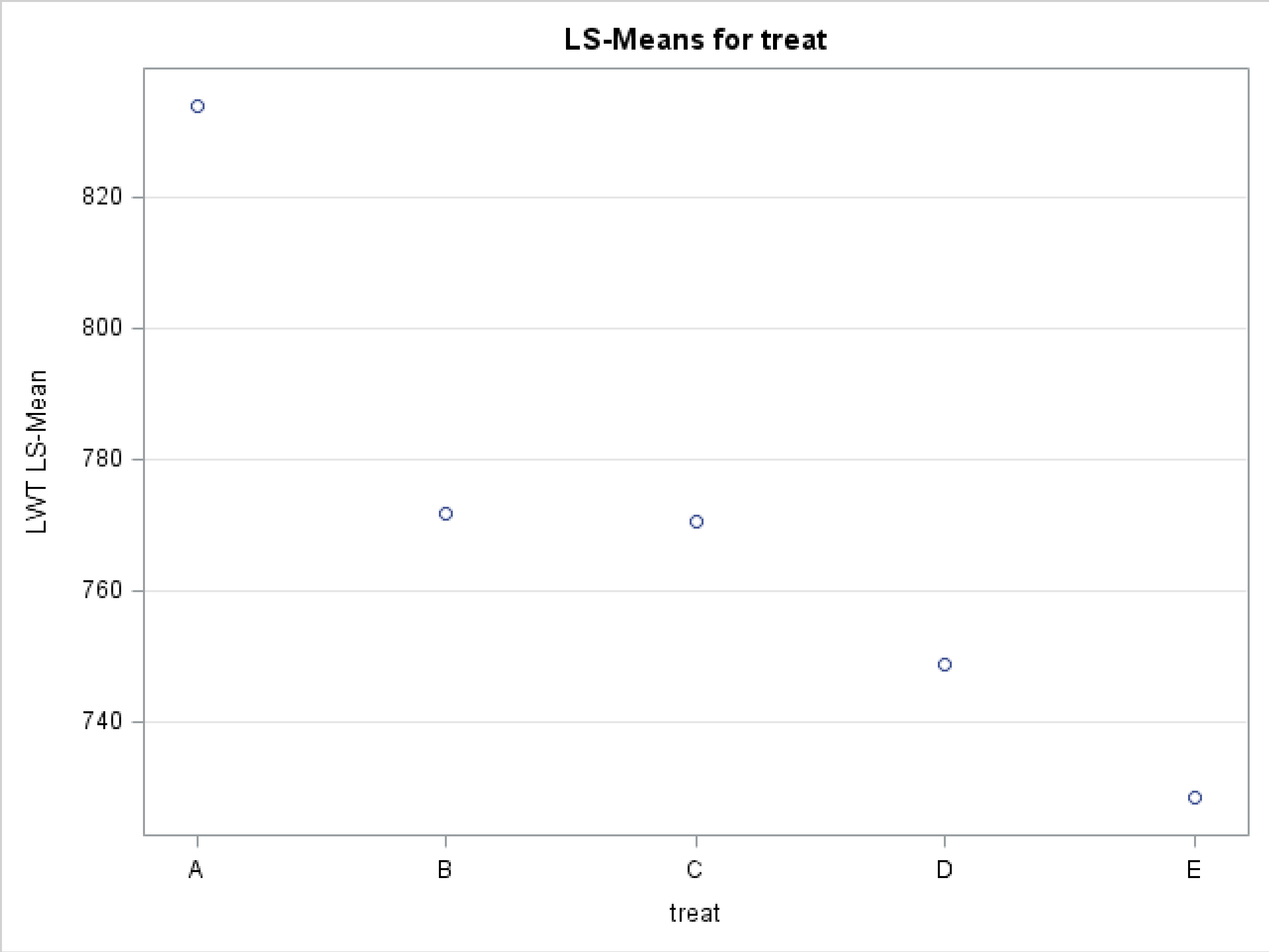
Tukey Grouping		**Mean**	**N**	**treat**
	A	3.000	5	A
	A			
B	A	1.000	5	D
B				
B		0.000	5	C
B				
B		0.000	5	B
B				
B		0.000	5	E

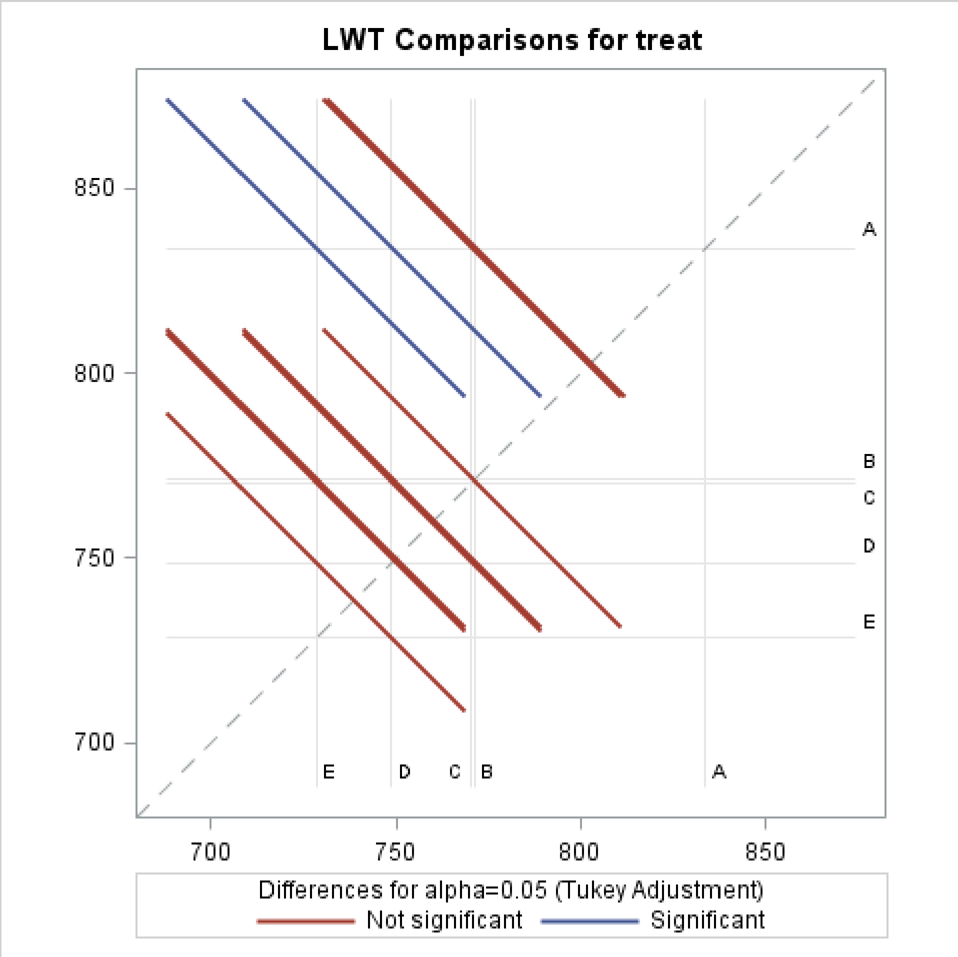
The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

treat	LWT LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	833.872000	19.116196	<.0001	1
B	771.560000	19.116196	<.0001	2
C	770.490000	19.116196	<.0001	3
D	748.720000	19.116196	<.0001	4
E	728.300000	19.116196	<.0001	5

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: LWT

i/j	1	2	3	4	5
1		0.1844	0.1722	0.0361	0.0070
2	0.1844		1.0000	0.9132	0.5141
3	0.1722	1.0000		0.9259	0.5377
4	0.0361	0.9132	0.9259		0.9403
5	0.0070	0.5141	0.5377	0.9403	


LS-Means for treat
LWT LS-Mean
820
800
780
760
740
A
B
C
D
E
treat



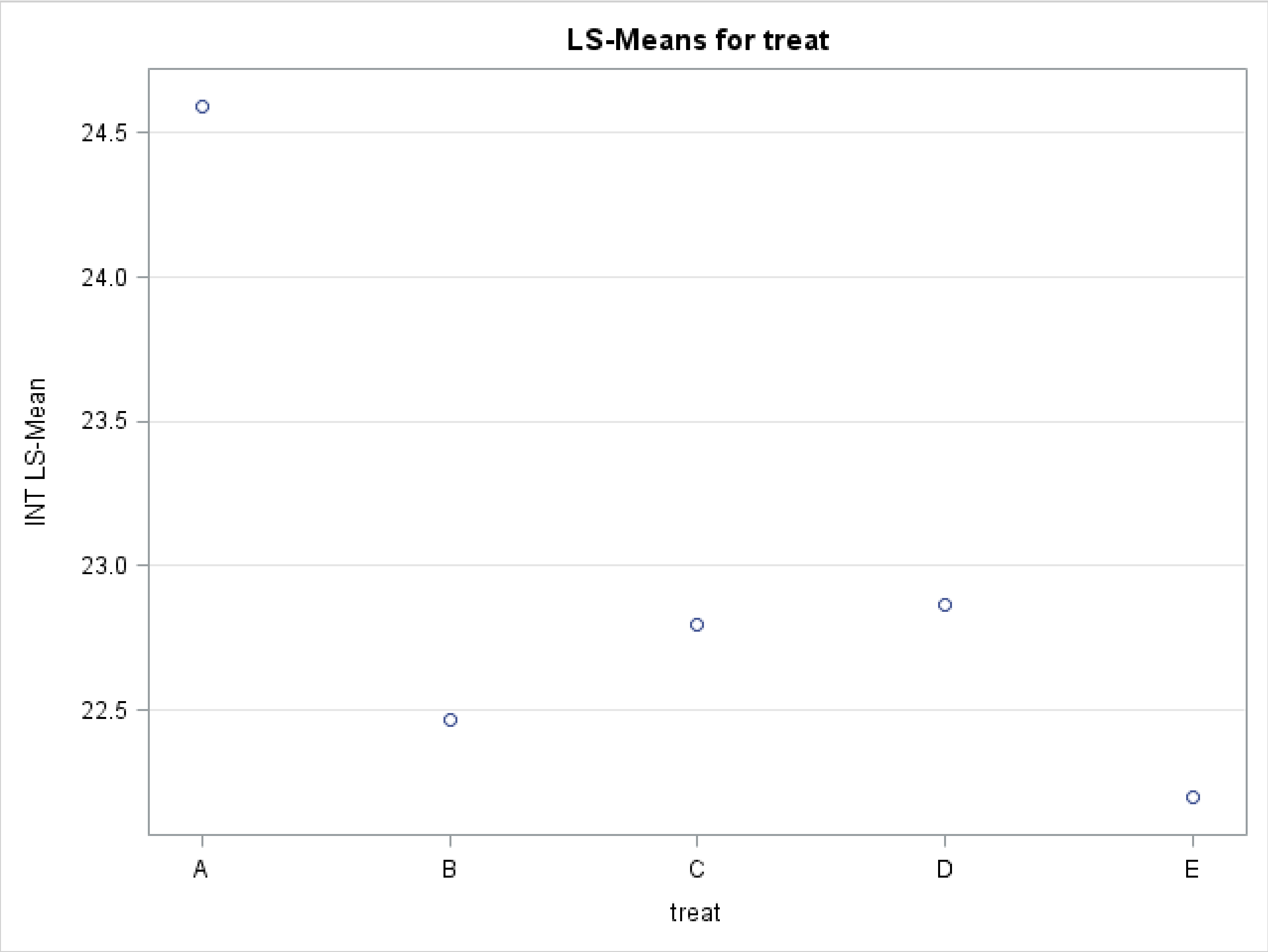
treat	INT LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	24.5920000	2.4418078	<.0001	1
B	22.4680000	2.4418078	<.0001	2
C	22.8000000	2.4418078	<.0001	3
D	22.8680000	2.4418078	<.0001	4
E	22.1980000	2.4418078	<.0001	5

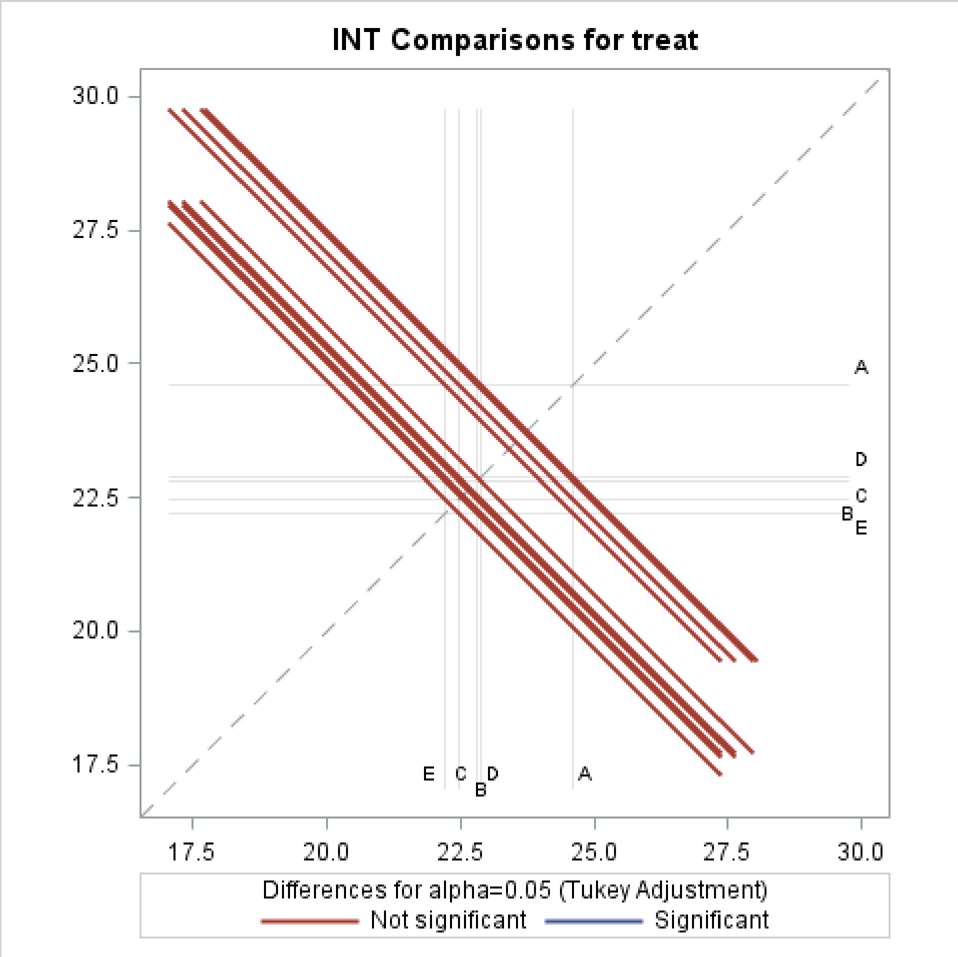
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: INT

i/j	1	2	3	4	5
1		0.9710	0.9844	0.9865	0.9556
2	0.9710		1.0000	1.0000	1.0000
3	0.9844	1.0000		1.0000	0.9998
4	0.9865	1.0000	1.0000		0.9997

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: INT

i/j	1	2	3	4	5
5	0.9556	1.0000	0.9998	0.9997	





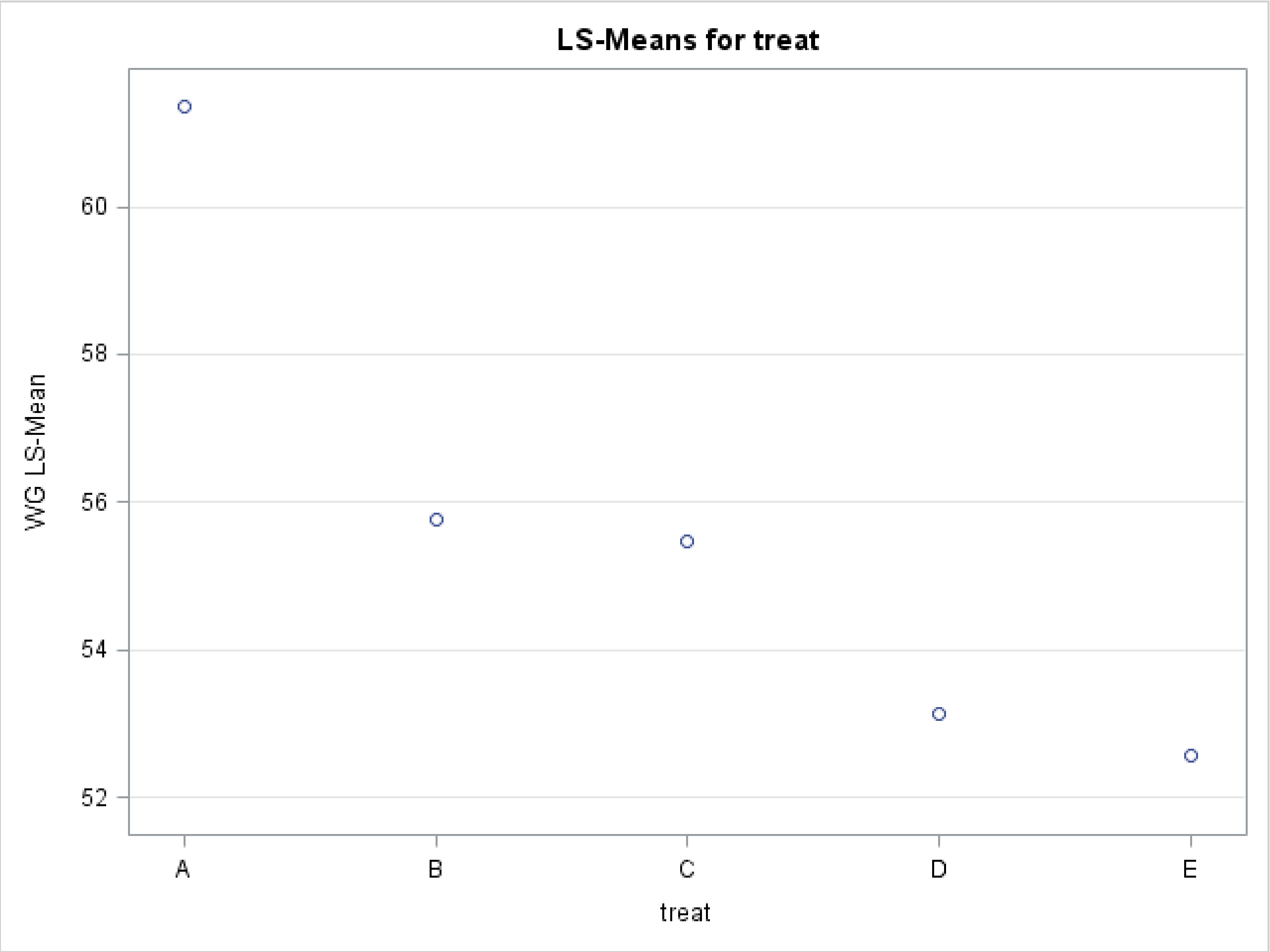
treat	WG LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	61.3600000	1.8231127	<.0001	1
B	55.7640000	1.8231127	<.0001	2
C	55.4620000	1.8231127	<.0001	3
D	53.1440000	1.8231127	<.0001	4
E	52.5620000	1.8231127	<.0001	5

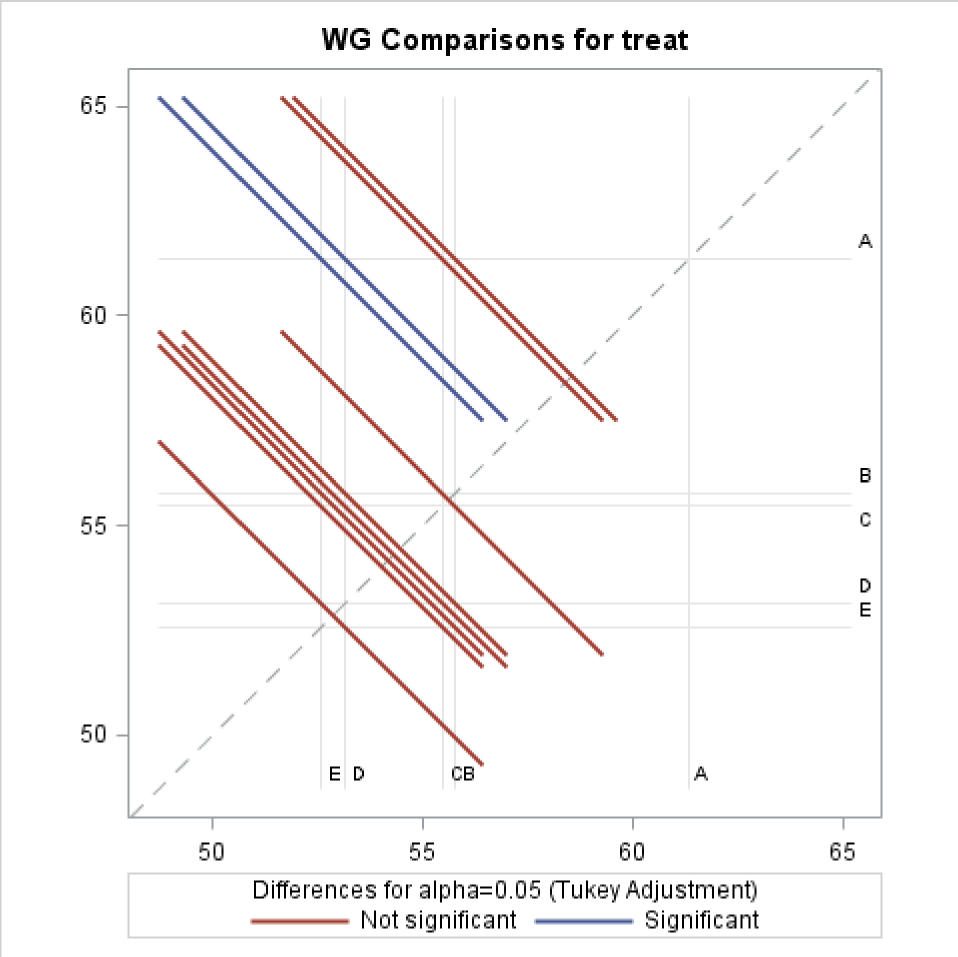
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: WG

i/j	1	2	3	4	5
1		0.2309	0.1899	0.0334	0.0206
2	0.2309		1.0000	0.8449	0.7278
3	0.1899	1.0000		0.8939	0.7918
4	0.0334	0.8449	0.8939		0.9994

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: WG

i/j	1	2	3	4	5
5	0.0206	0.7278	0.7918	0.9994	





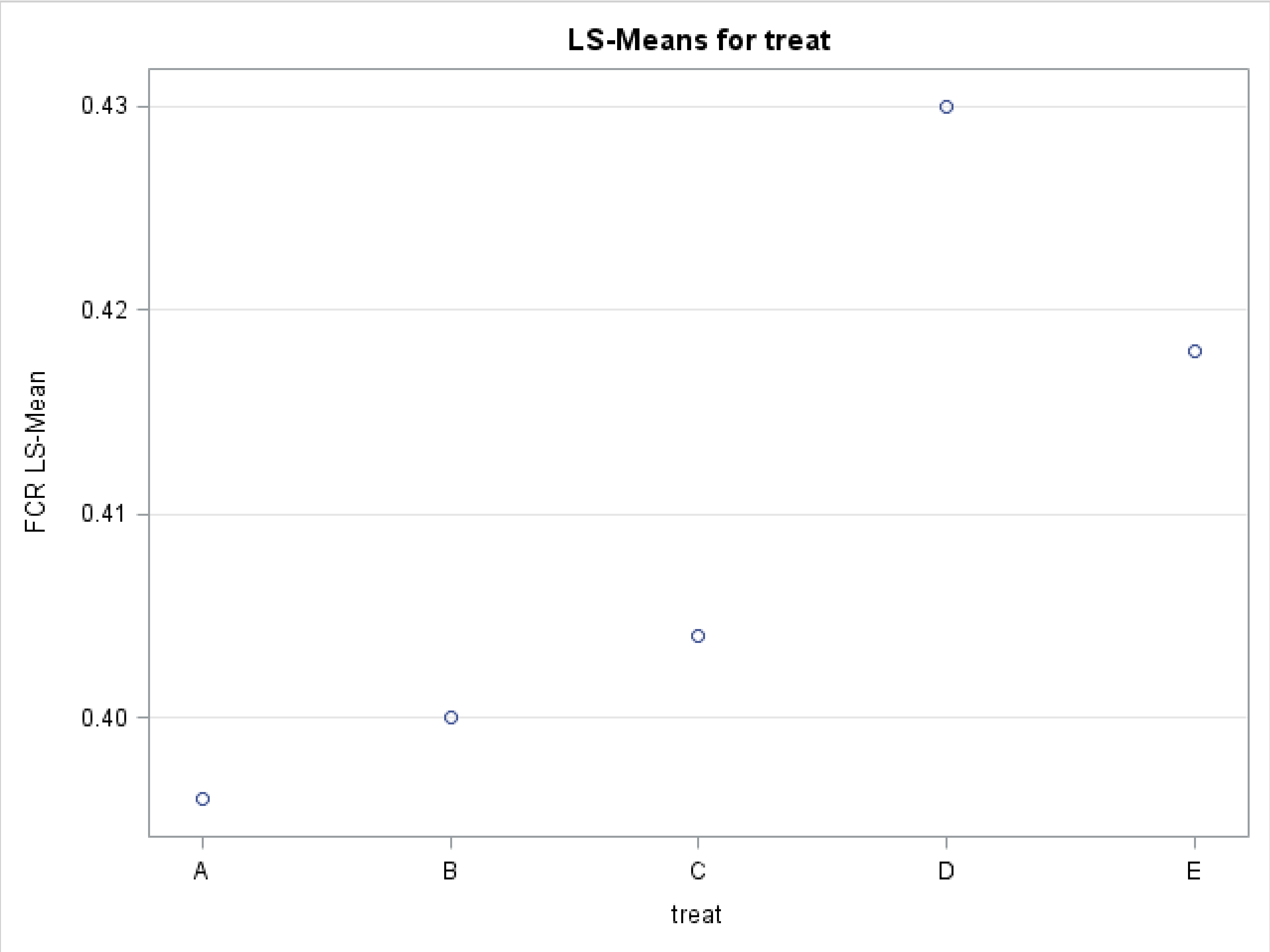
treat	FCR LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	0.39600000	0.04232257	<.0001	1
B	0.40000000	0.04232257	<.0001	2
C	0.40400000	0.04232257	<.0001	3
D	0.43000000	0.04232257	<.0001	4
E	0.41800000	0.04232257	<.0001	5

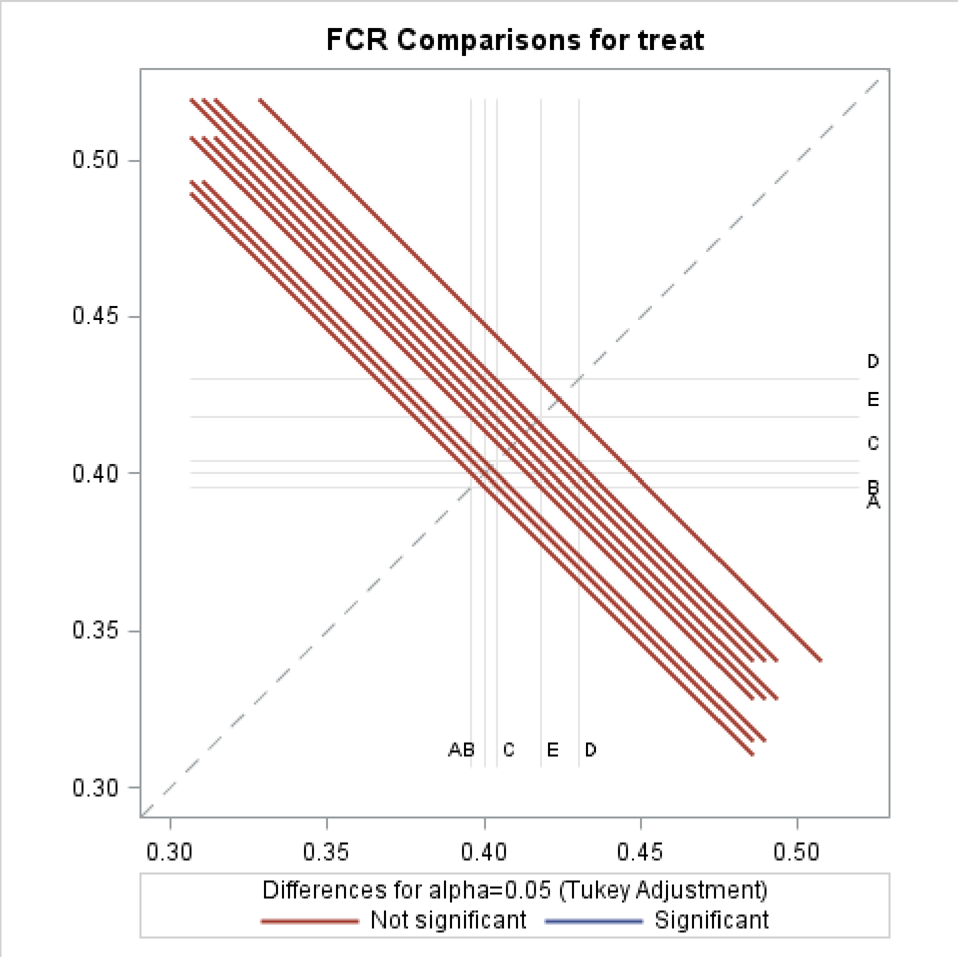
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: FCR

i/j	1	2	3	4	5
1		1.0000	0.9999	0.9783	0.9958
2	1.0000		1.0000	0.9863	0.9981
3	0.9999	1.0000		0.9920	0.9993
4	0.9783	0.9863	0.9920		0.9996

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: FCR

i/j	1	2	3	4	5
5	0.9958	0.9981	0.9993	0.9996	





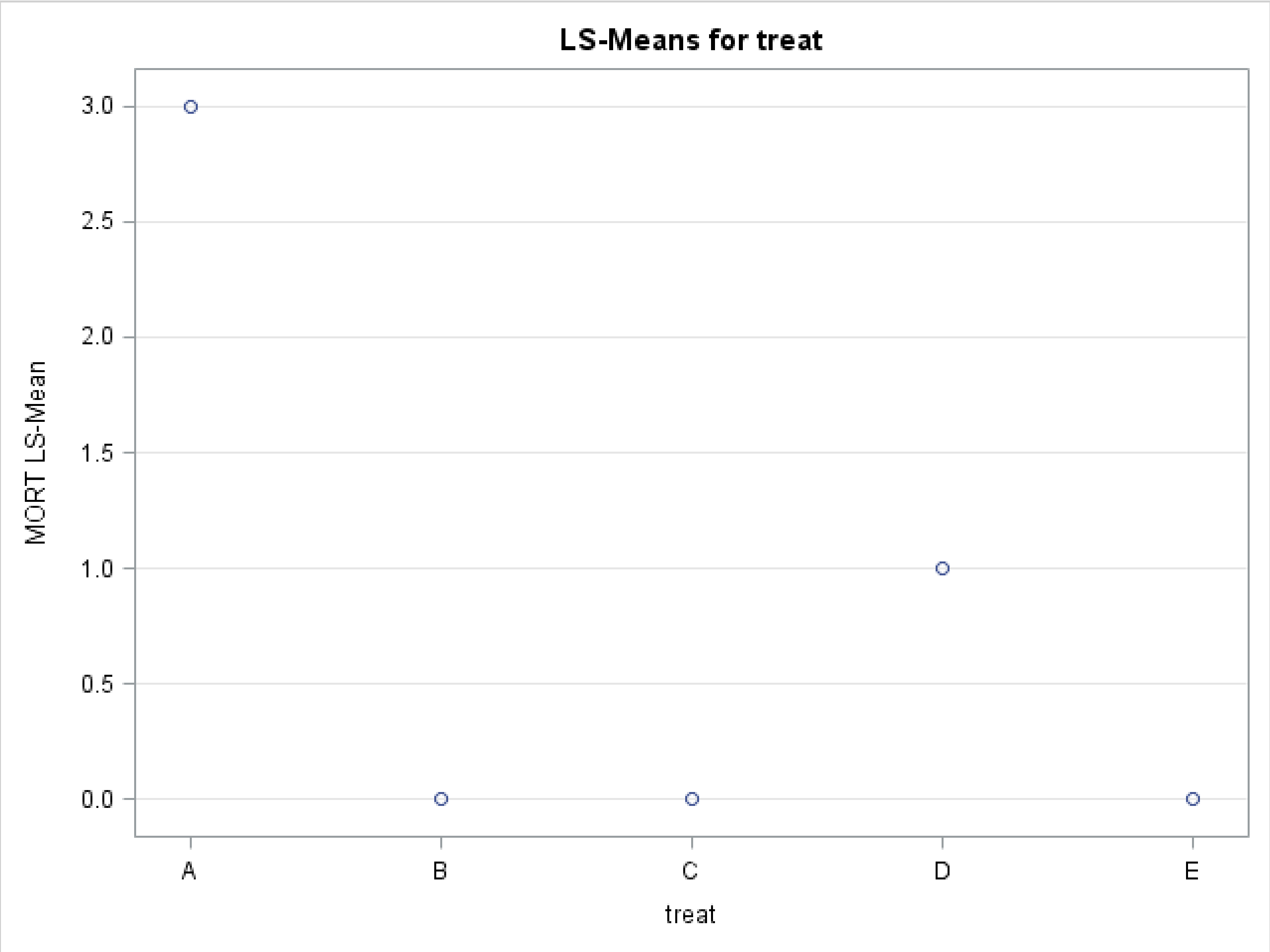
treat	MORT LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	3.00000000	0.70710678	0.0004	1
B	0.00000000	0.70710678	1.0000	2
C	0.00000000	0.70710678	1.0000	3
D	1.00000000	0.70710678	0.1727	4
E	0.00000000	0.70710678	1.0000	5

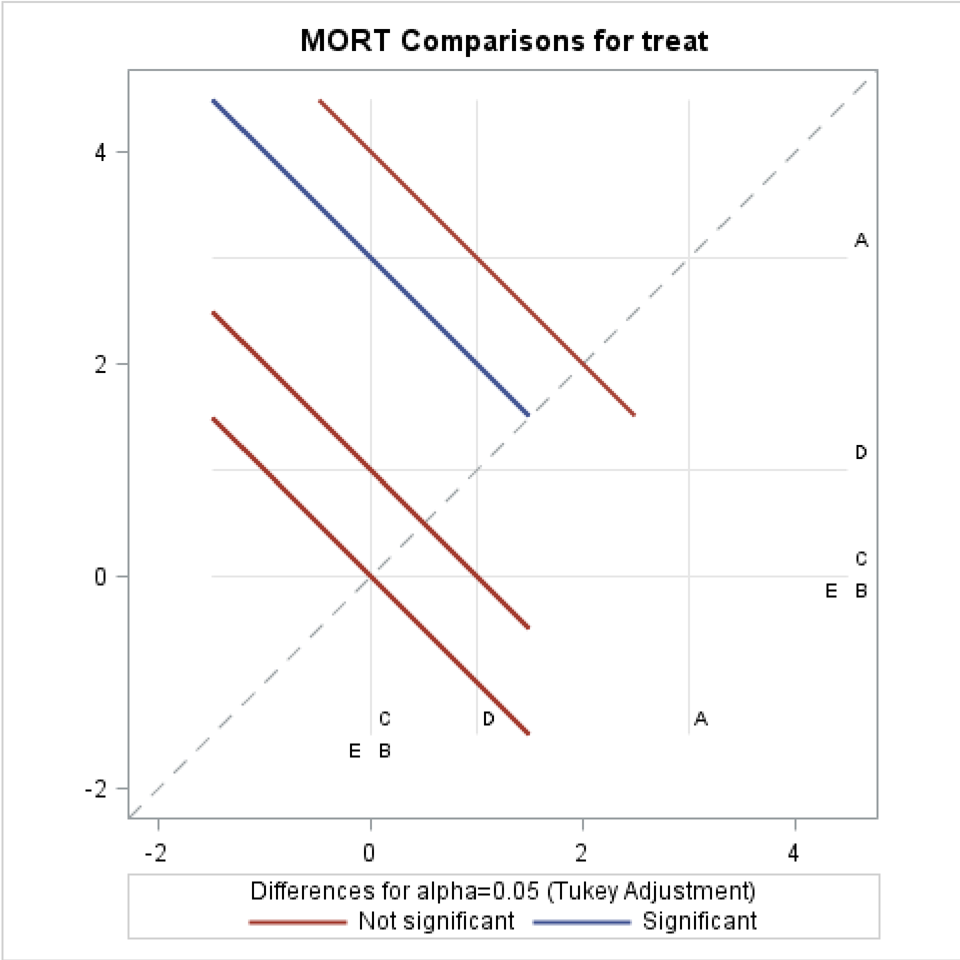
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: MORT

i/j	1	2	3	4	5
1		0.0492	0.0492	0.3016	0.0492
2	0.0492		1.0000	0.8523	1.0000
3	0.0492	1.0000		0.8523	1.0000
4	0.3016	0.8523	0.8523		0.8523

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: MORT

i/j	1	2	3	4	5
5	0.0492	1.0000	1.0000	0.8523	




MORT Comparisons for treat
4
2
0
-2
-2
0
2
4
A
D
C
E B
C
D
A
E B
Differences for alpha=0.05 (Tukey Adjustment)
Not significant
Significant

The GLM Procedure

Class Level Information

Class	Levels	Values
treat	5	A B C D E

Number of Observations Read	25
Number of Observations Used	25

The SAS System

The GLM Procedure

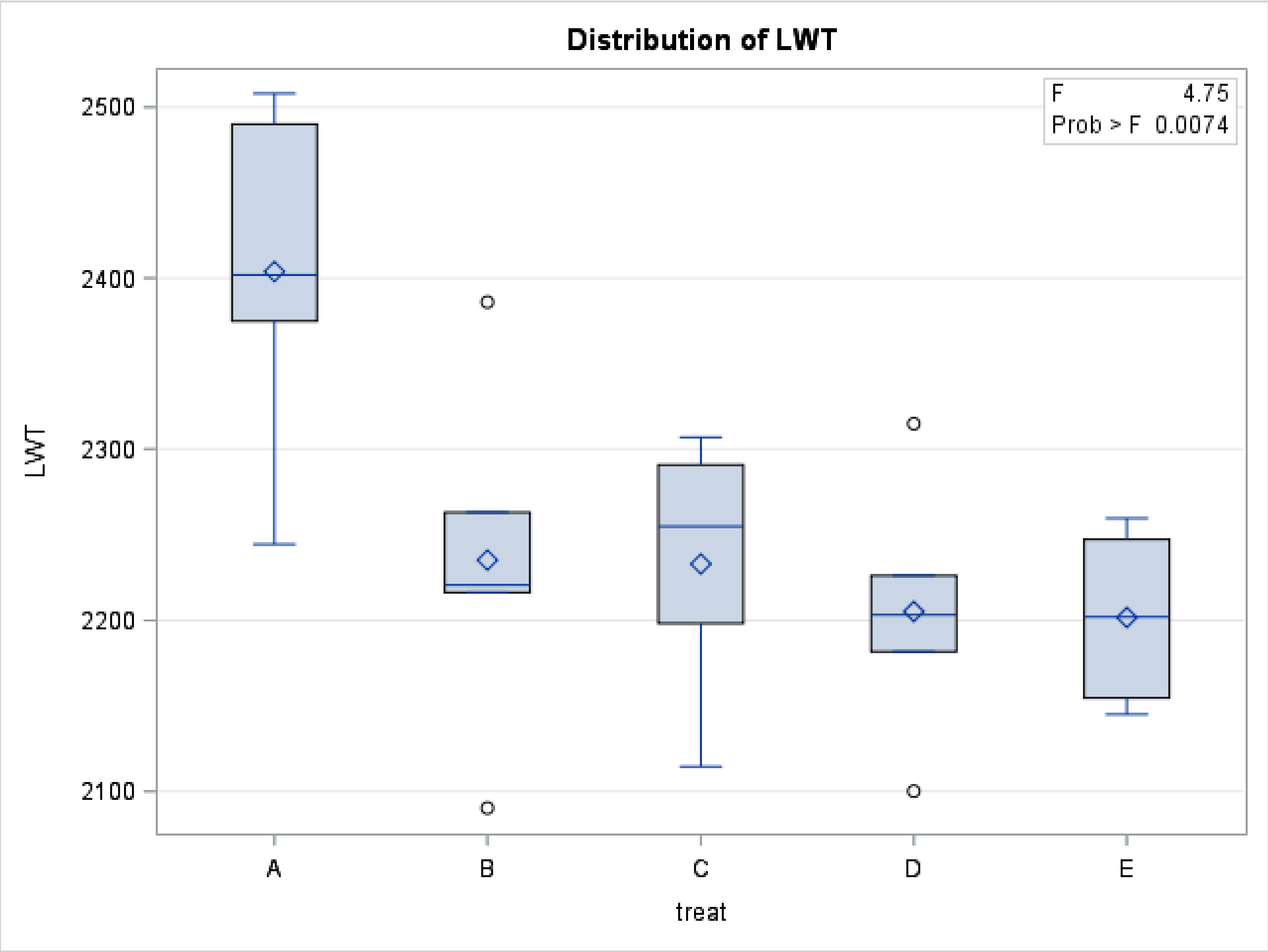
Dependent Variable: LWT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	141683.6479	35420.9120	4.75	0.0074
Error	20	149056.3540	7452.8177		
Corrected Total	24	290740.0019			

R-Square	Coeff Var	Root MSE	LWT Mean
0.487321	3.826925	86.32970	2255.850

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	141683.6479	35420.9120	4.75	0.0074

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	141683.6479	35420.9120	4.75	0.0074



The GLM Procedure

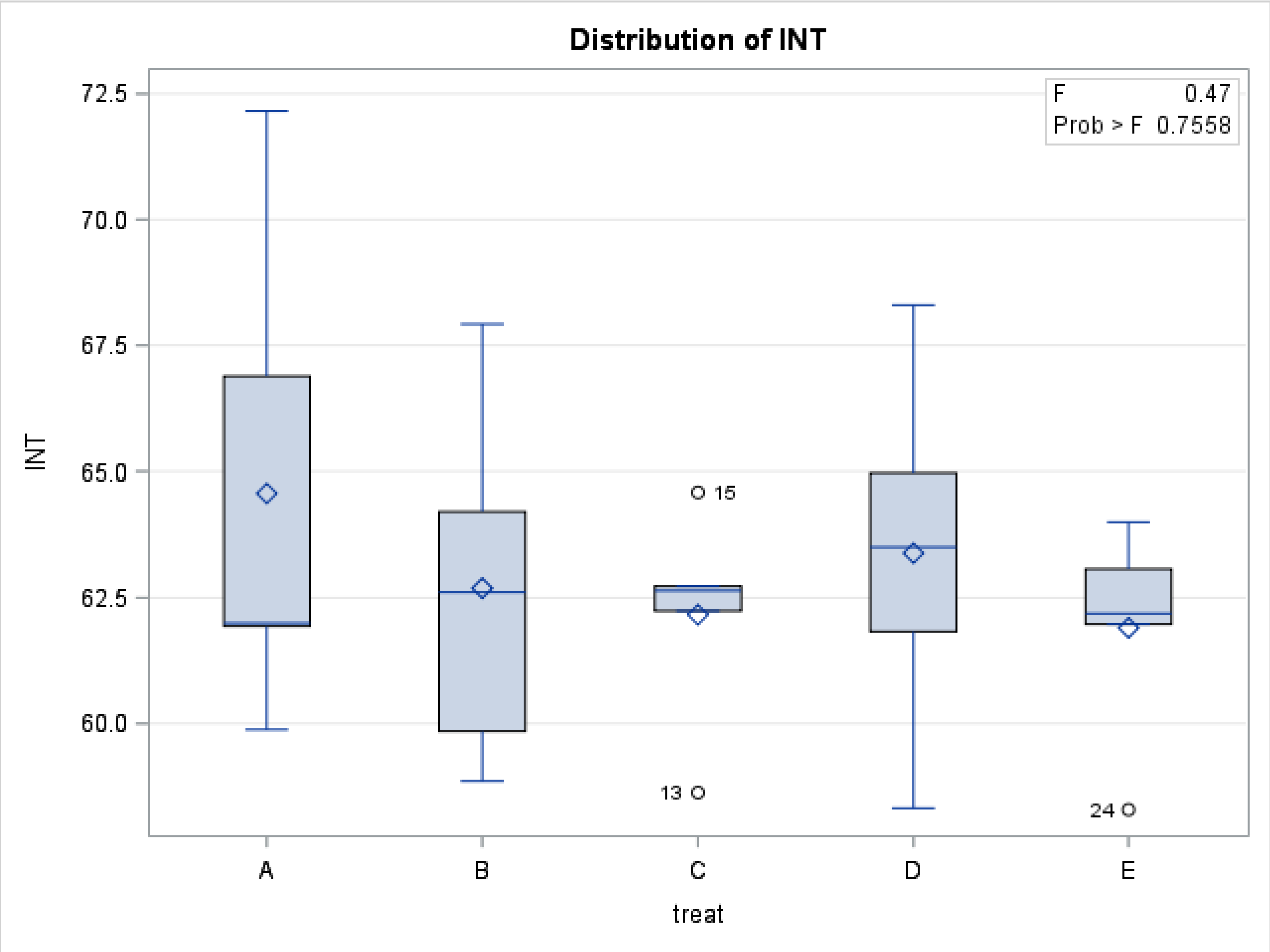
Dependent Variable: INT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	22.9799360	5.7449840	0.47	0.7558
Error	20	243.4644400	12.1732220		
Corrected Total	24	266.4443760			

R-Square	Coeff Var	Root MSE	INT Mean
0.086247	5.543081	3.489014	62.94360

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	22.97993600	5.74498400	0.47	0.7558

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	22.97993600	5.74498400	0.47	0.7558



The SAS System

The GLM Procedure

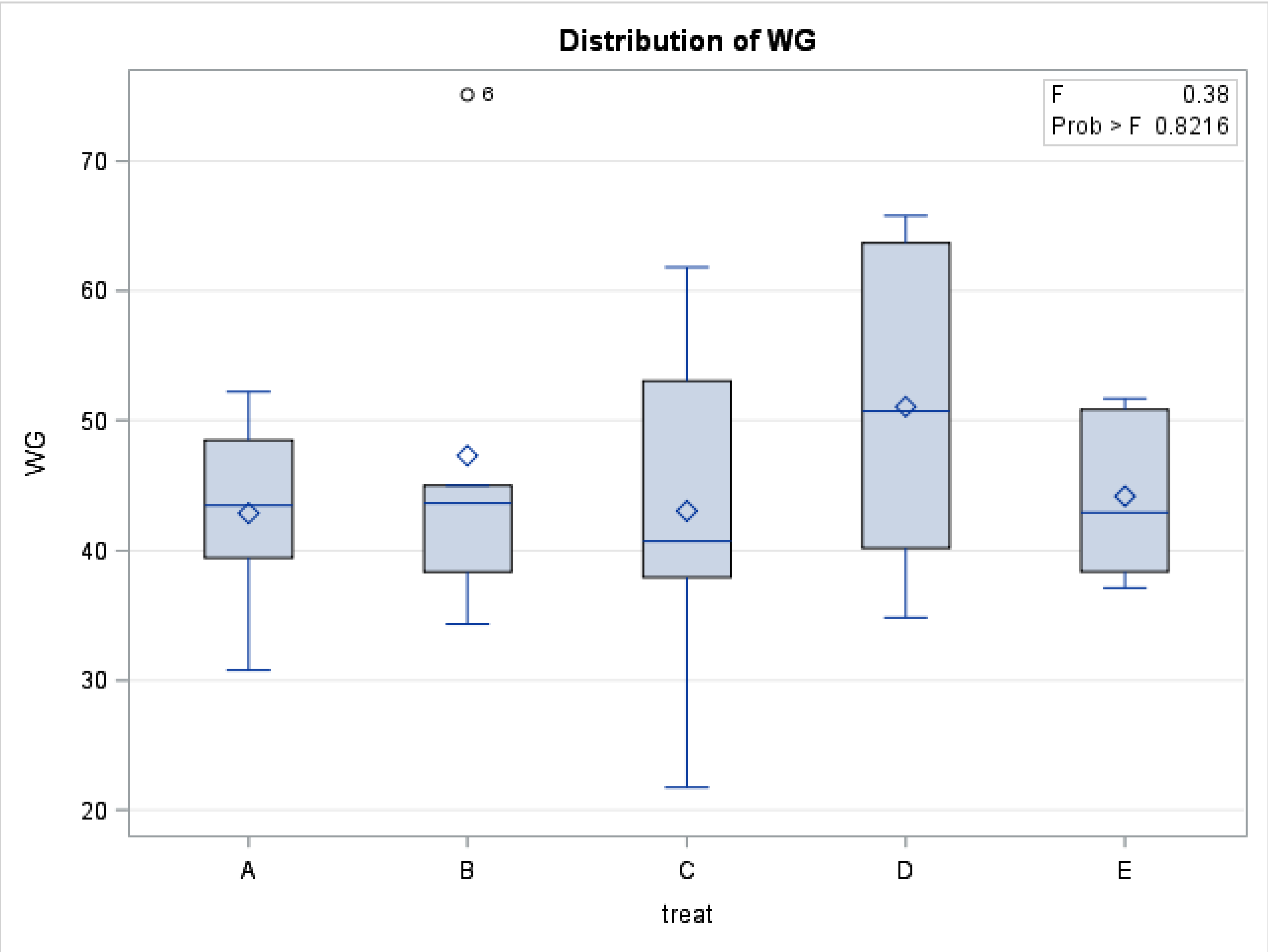
Dependent Variable: WG

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	242.082760	60.520690	0.38	0.8216
Error	20	3201.387440	160.069372		
Corrected Total	24	3443.470200			

R-Square	Coeff Var	Root MSE	WG Mean
0.070302	27.68579	12.65185	45.69800

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	242.0827600	60.5206900	0.38	0.8216

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	242.0827600	60.5206900	0.38	0.8216



The GLM Procedure

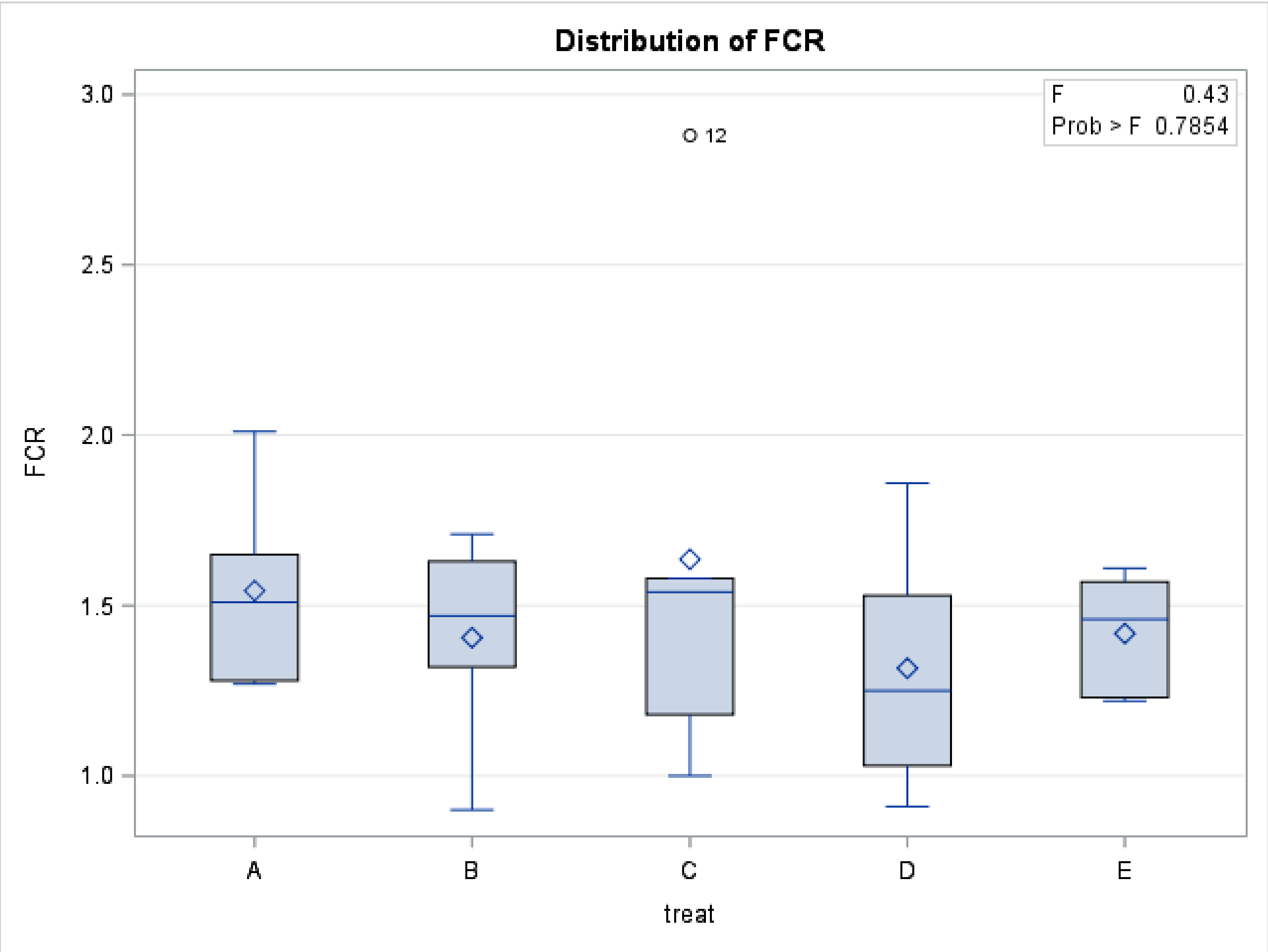
Dependent Variable: FCR

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.31684000	0.07921000	0.43	0.7854
Error	20	3.68576000	0.18428800		
Corrected Total	24	4.00260000			

R-Square	Coeff Var	Root MSE	FCR Mean
0.079159	29.32294	0.429288	1.464000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	0.31684000	0.07921000	0.43	0.7854

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	0.31684000	0.07921000	0.43	0.7854



The SAS System

The GLM Procedure

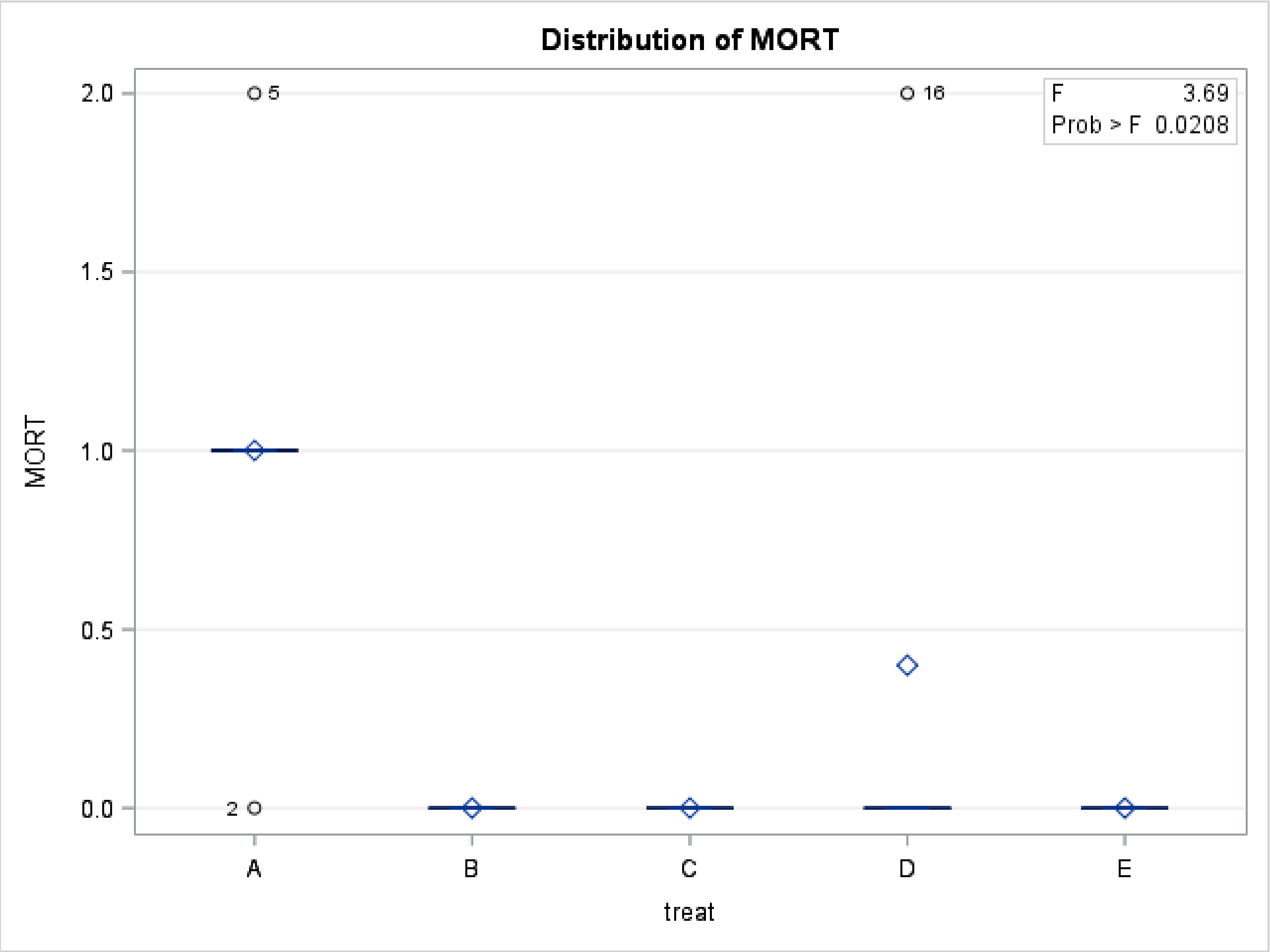
Dependent Variable: MORT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3.84000000	0.96000000	3.69	0.0208
Error	20	5.20000000	0.26000000		
Corrected Total	24	9.04000000			

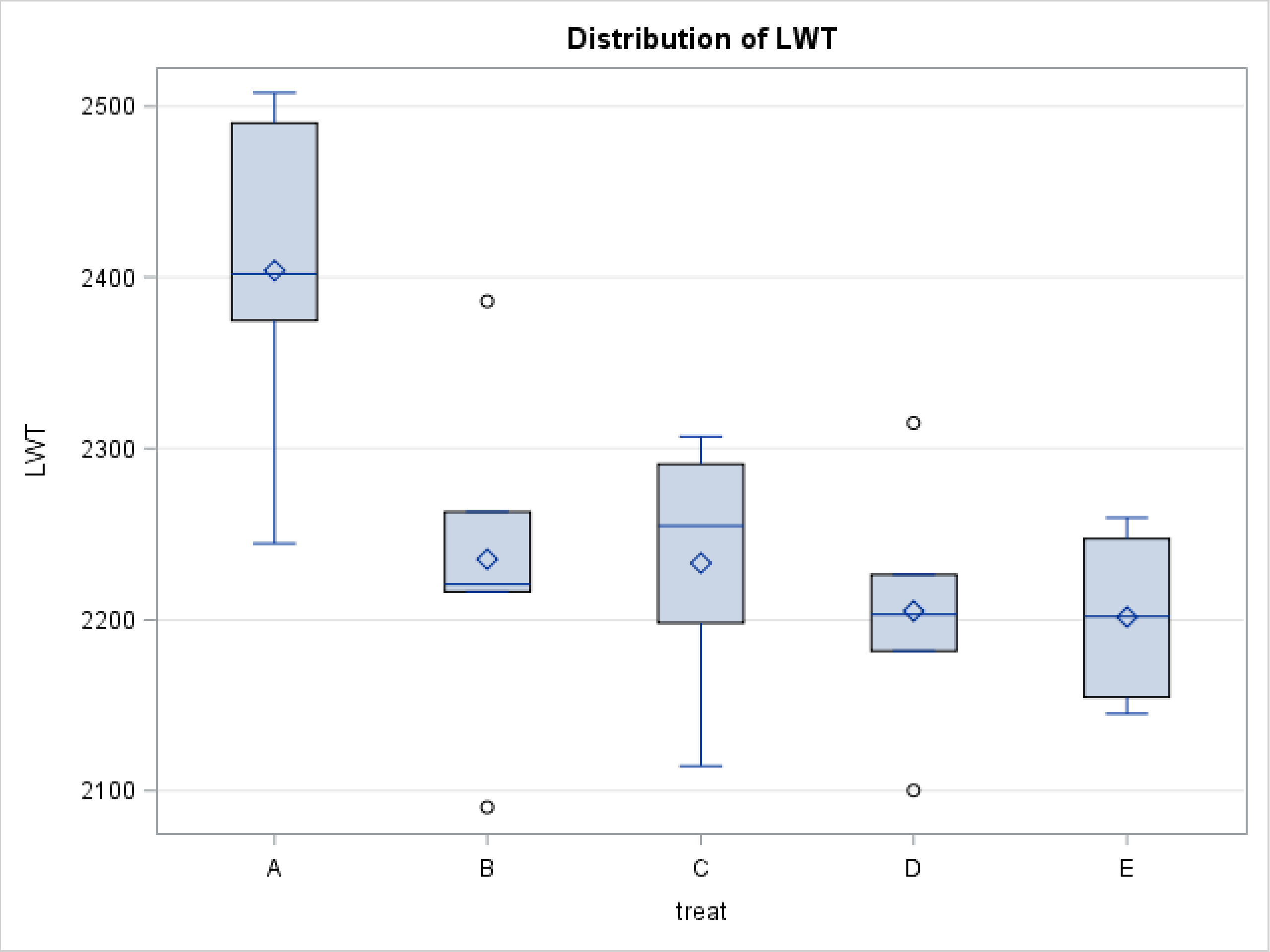
R-Square	Coeff Var	Root MSE	MORT Mean
0.424779	182.1078	0.509902	0.280000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	3.84000000	0.96000000	3.69	0.0208

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	3.84000000	0.96000000	3.69	0.0208



The GLM Procedure



The SAS System

The GLM Procedure

t Tests (LSD) for LWT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	7452.818
Critical Value of t	2.08596
Least Significant Difference	113.89

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	2403.87	5	A
B	2235.30	5	B
B			
B	2233.08	5	C
B			
B	2205.23	5	D
B			
B	2201.77	5	E

The GLM Procedure

Duncan's Multiple Range Test for LWT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	7452.818

Number of Means	**2**	**3**	**4**	**5**
Critical Range	113.9	119.5	123.1	125.7

Means with the same letter are not significantly different.

Duncan Grouping	**Mean**	**N**	**treat**
A	2403.87	5	A
B	2235.30	5	B
B			
B	2233.08	5	C
B			
B	2205.23	5	D
B			
B	2201.77	5	E

The GLM Procedure

Tukey's Studentized Range (HSD) Test for LWT

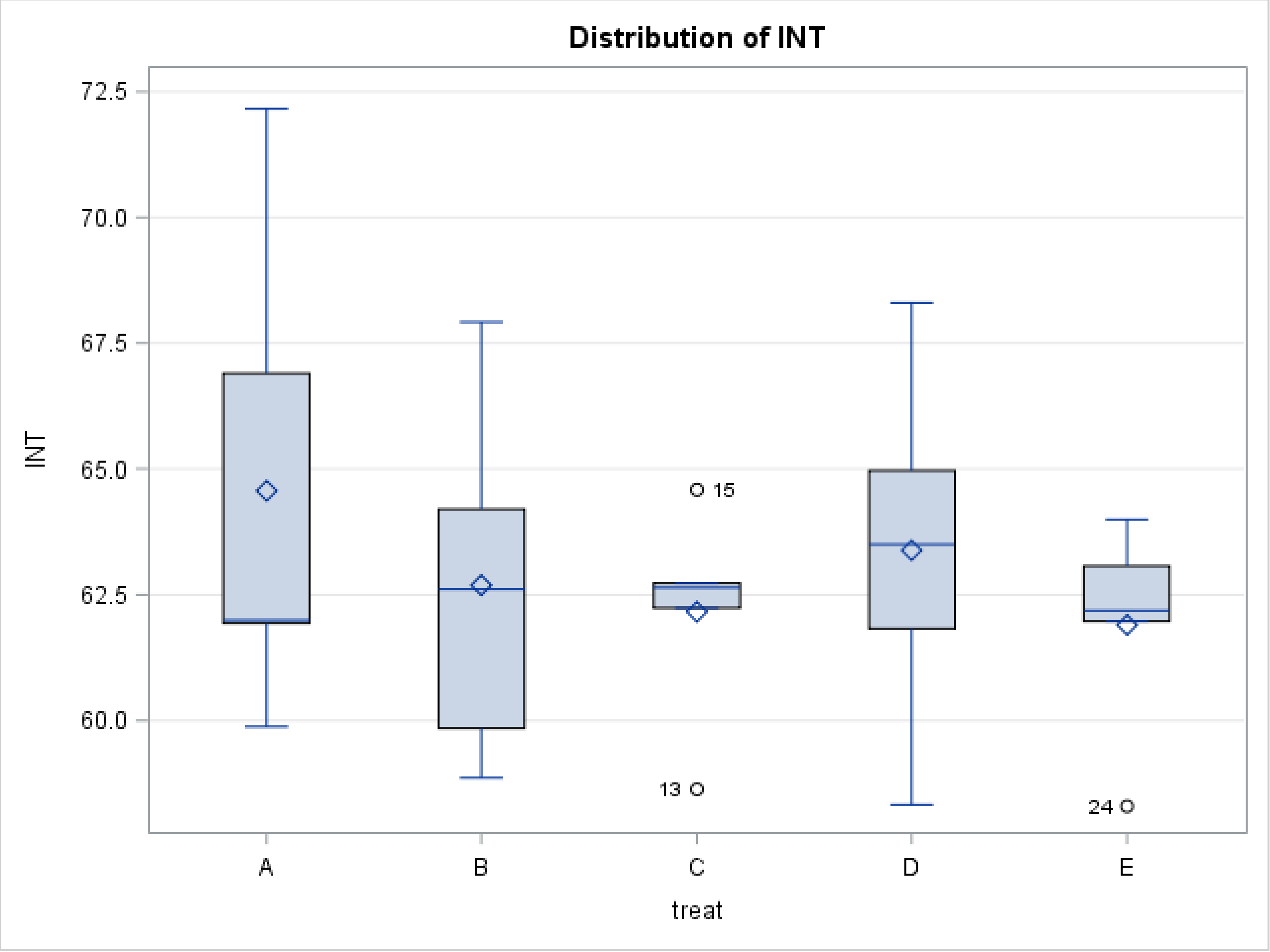
Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	7452.818
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	163.38

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	2403.87	5	A
B	2235.30	5	B
B			
B	2233.08	5	C
B			
B	2205.23	5	D
B			
B	2201.77	5	E

The GLM Procedure



The SAS System

The GLM Procedure

t Tests (LSD) for INT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	12.17322
Critical Value of t	2.08596
Least Significant Difference	4.603

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	64.574	5	A
A			
A	63.382	5	D
A			
A	62.690	5	B
A			
A	62.166	5	C
A			
A	61.906	5	E

The GLM Procedure

Duncan's Multiple Range Test for INT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	12.17322

Number of Means	**2**	**3**	**4**	**5**
Critical Range	4.603	4.832	4.977	5.078

Means with the same letter are not significantly different.

Duncan Grouping	**Mean**	**N**	**treat**
A	64.574	5	A
A			
A	63.382	5	D
A			
A	62.690	5	B
A			
A	62.166	5	C
A			
A	61.906	5	E

The GLM Procedure

Tukey's Studentized Range (HSD) Test for INT

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

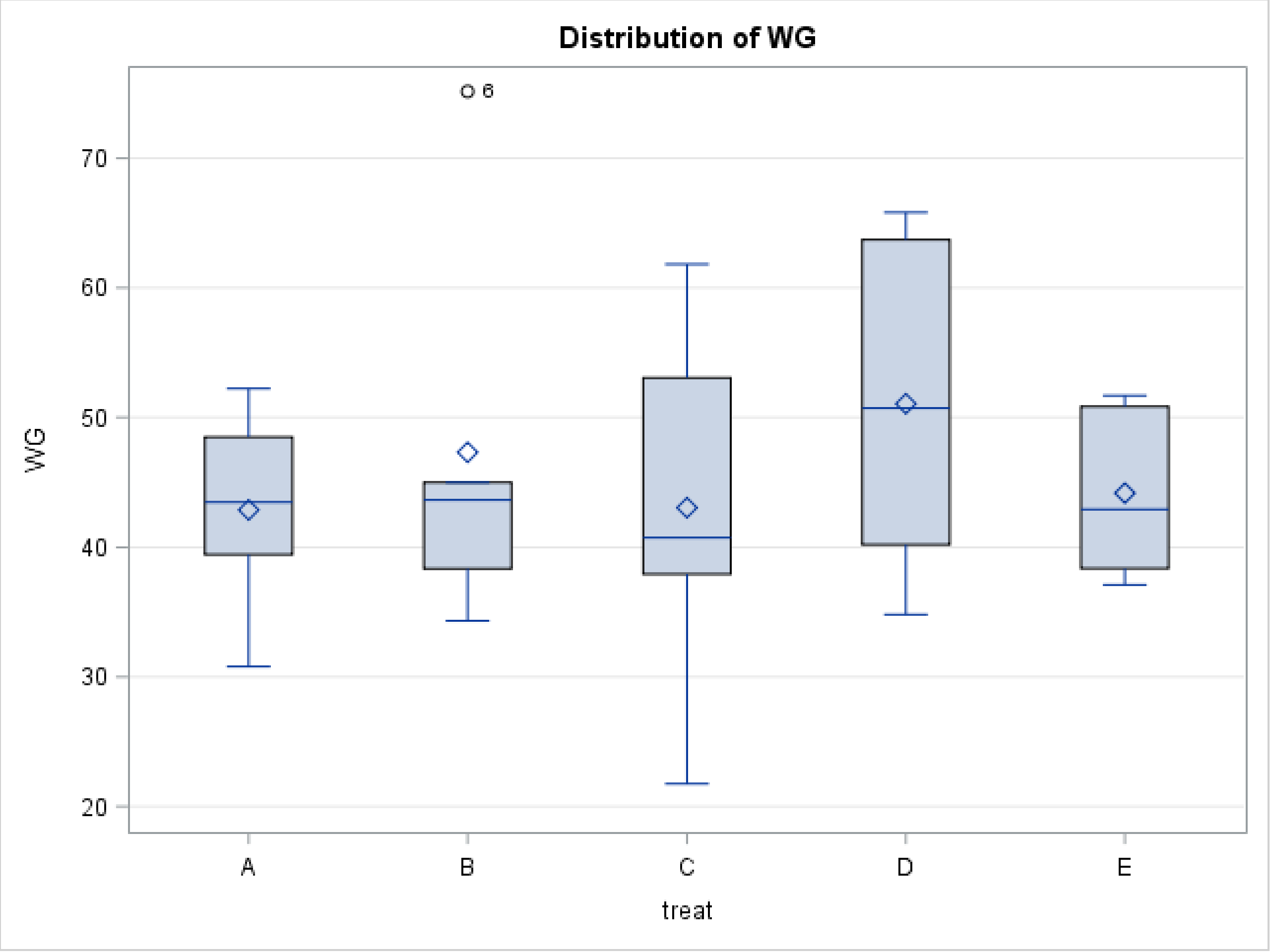
Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	12.17322
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	6.6031

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	64.574	5	A
A			
A	63.382	5	D
A			
A	62.690	5	B
A			
A	62.166	5	C
A			
A	61.906	5	E

The SAS System

The GLM Procedure



The SAS System

The GLM Procedure

t Tests (LSD) for WG

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	160.0694
Critical Value of t	2.08596
Least Significant Difference	16.691

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	51.056	5	D
A			
A	47.300	5	B
A			
A	44.186	5	E
A			
A	43.062	5	C
A			
A	42.886	5	A

The GLM Procedure

Duncan's Multiple Range Test for WG

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	160.0694

Number of Means	**2**	**3**	**4**	**5**
Critical Range	16.69	17.52	18.05	18.41

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treat
A	51.056	5	D
A			
A	47.300	5	B
A			
A	44.186	5	E
A			
A	43.062	5	C
A			
A	42.886	5	A

The GLM Procedure

Tukey's Studentized Range (HSD) Test for WG

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

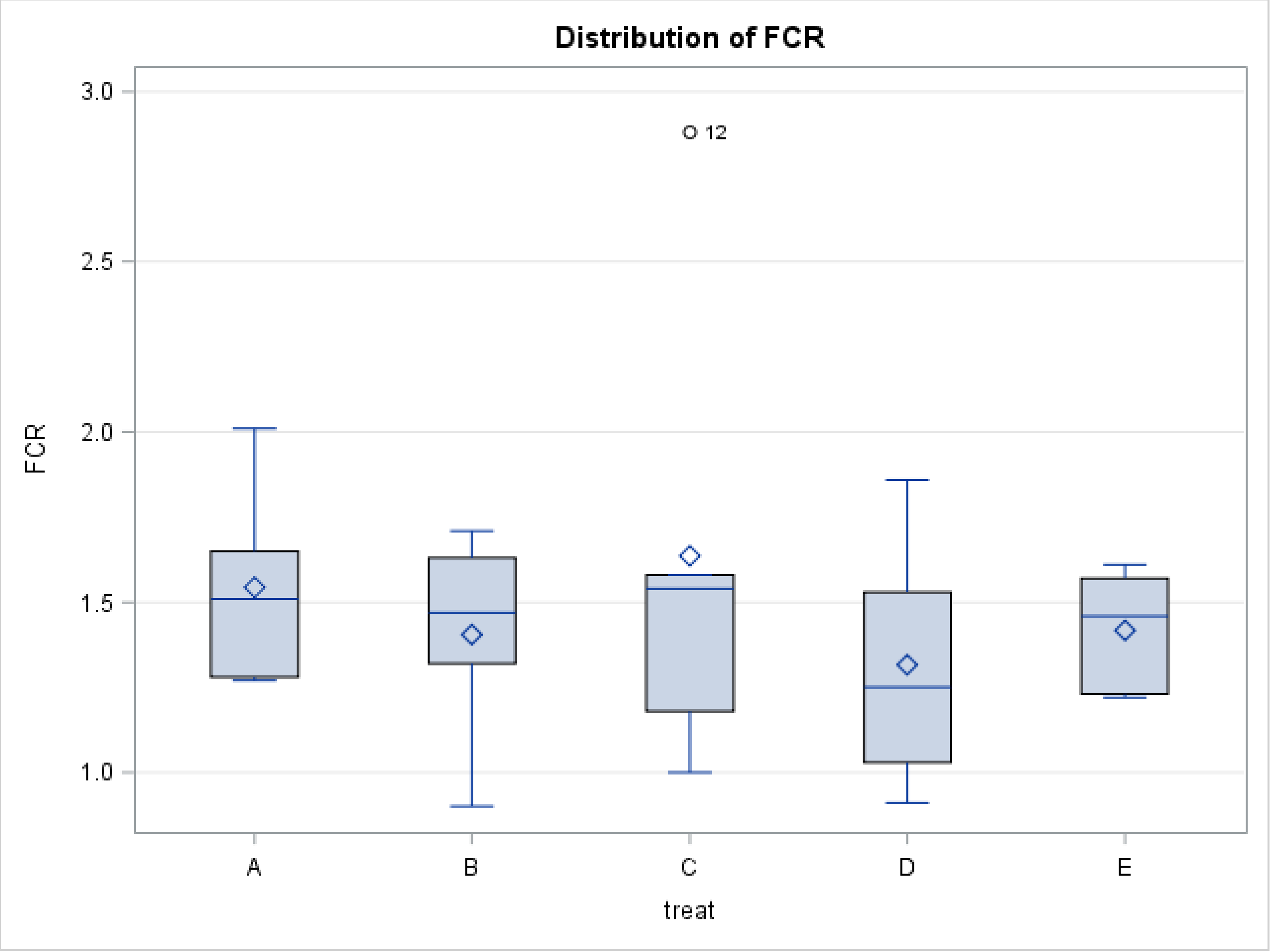
Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	160.0694
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	23.944

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	51.056	5	D
A			
A	47.300	5	B
A			
A	44.186	5	E
A			
A	43.062	5	C
A			
A	42.886	5	A

The SAS System

The GLM Procedure



The SAS System

The GLM Procedure

t Tests (LSD) for FCR

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.184288
Critical Value of t	2.08596
Least Significant Difference	0.5664

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	1.6360	5	C
A			
A	1.5440	5	A
A			
A	1.4180	5	E
A			
A	1.4060	5	B
A			
A	1.3160	5	D

The GLM Procedure

Duncan's Multiple Range Test for FCR

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.184288

Number of Means	**2**	**3**	**4**	**5**
Critical Range	.5663	.5945	.6124	.6248

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treat
A	1.6360	5	C
A			
A	1.5440	5	A
A			
A	1.4180	5	E
A			
A	1.4060	5	B
A			
A	1.3160	5	D

The SAS System

The GLM Procedure

Tukey's Studentized Range (HSD) Test for FCR

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

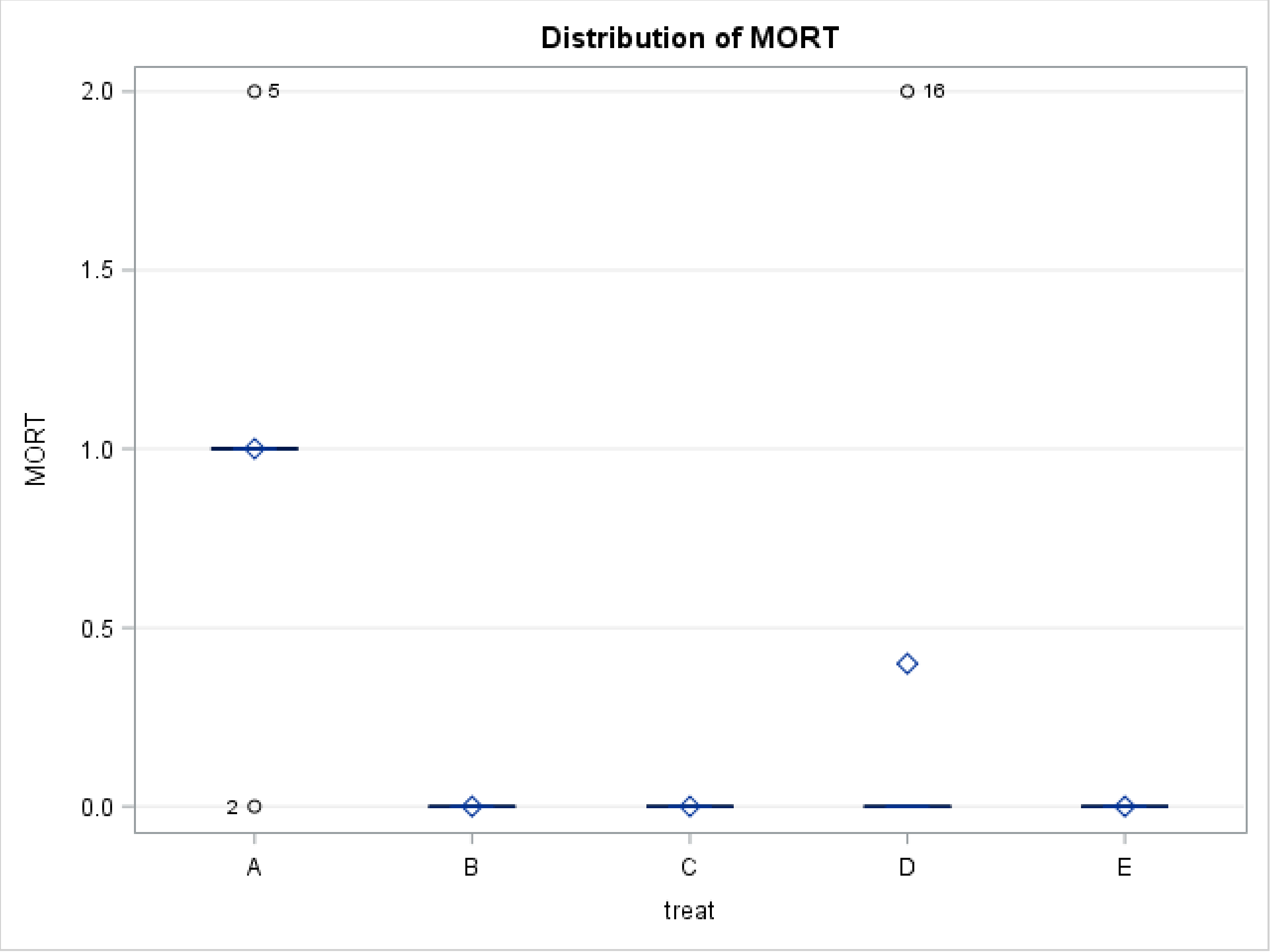
Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.184288
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.8124

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	1.6360	5	C
A			
A	1.5440	5	A
A			
A	1.4180	5	E
A			
A	1.4060	5	B
A			
A	1.3160	5	D

The SAS System

The GLM Procedure



The GLM Procedure

t Tests (LSD) for MORT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.26
Critical Value of t	2.08596
Least Significant Difference	0.6727

Means with the same letter are not significantly different.

t Grouping		Mean	N	treat
	A	1.0000	5	A
	A			
B	A	0.4000	5	D
B				
B		0.0000	5	C
B				
B		0.0000	5	B
B				
B		0.0000	5	E

The GLM Procedure

Duncan's Multiple Range Test for MORT

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.26

Number of Means	**2**	**3**	**4**	**5**
Critical Range	.6727	.7061	.7273	.7422

Means with the same letter are not significantly different.

Duncan Grouping		**Mean**	**N**	**treat**
	A	1.0000	5	A
	A			
B	A	0.4000	5	D
B				
B		0.0000	5	C
B				
B		0.0000	5	B
B				
B		0.0000	5	E

The SAS System

The GLM Procedure

Tukey's Studentized Range (HSD) Test for MORT

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.26
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.965

Means with the same letter are not significantly different.

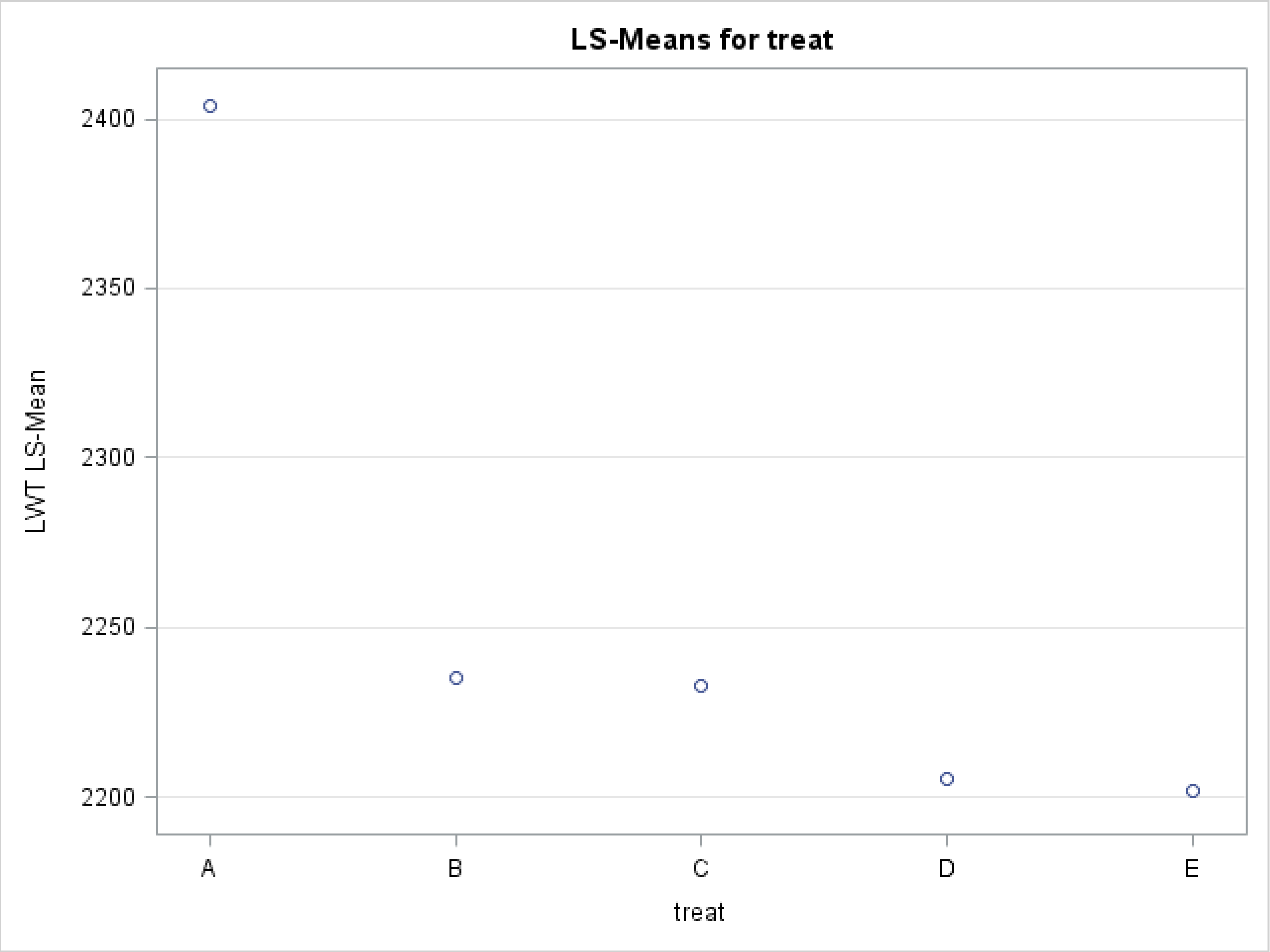
Tukey Grouping		**Mean**	**N**	**treat**
	A	1.0000	5	A
	A			
B	A	0.4000	5	D
B				
B		0.0000	5	C
B				
B		0.0000	5	B
B				
B		0.0000	5	E

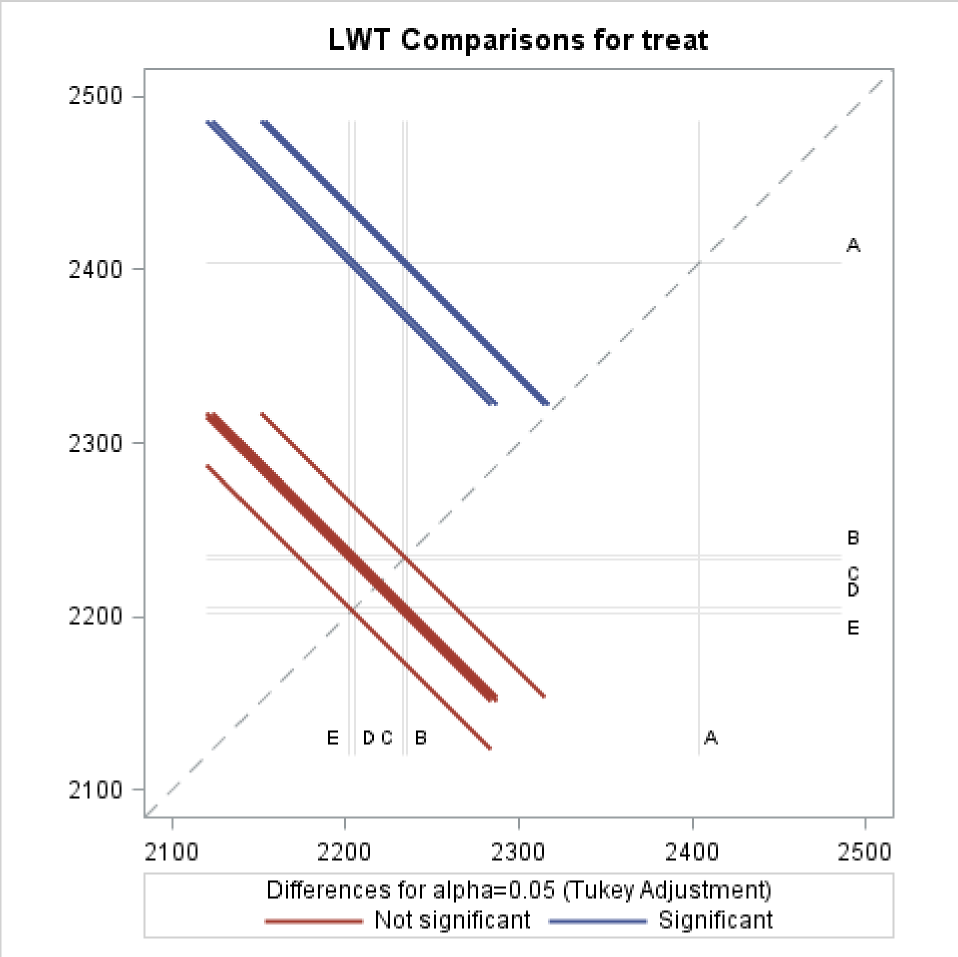
The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

treat	LWT LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	2403.86800	38.60782	<.0001	1
B	2235.30000	38.60782	<.0001	2
C	2233.08000	38.60782	<.0001	3
D	2205.23400	38.60782	<.0001	4
E	2201.77000	38.60782	<.0001	5

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: LWT

i/j	1	2	3	4	5
1		0.0411	0.0378	0.0126	0.0110
2	0.0411		1.0000	0.9806	0.9711
3	0.0378	1.0000		0.9854	0.9775
4	0.0126	0.9806	0.9854		1.0000
5	0.0110	0.9711	0.9775	1.0000	


LS-Means for treat
LWT LS-Mean
2400
2350
2300
2250
2200
A
B
C
D
E
treat



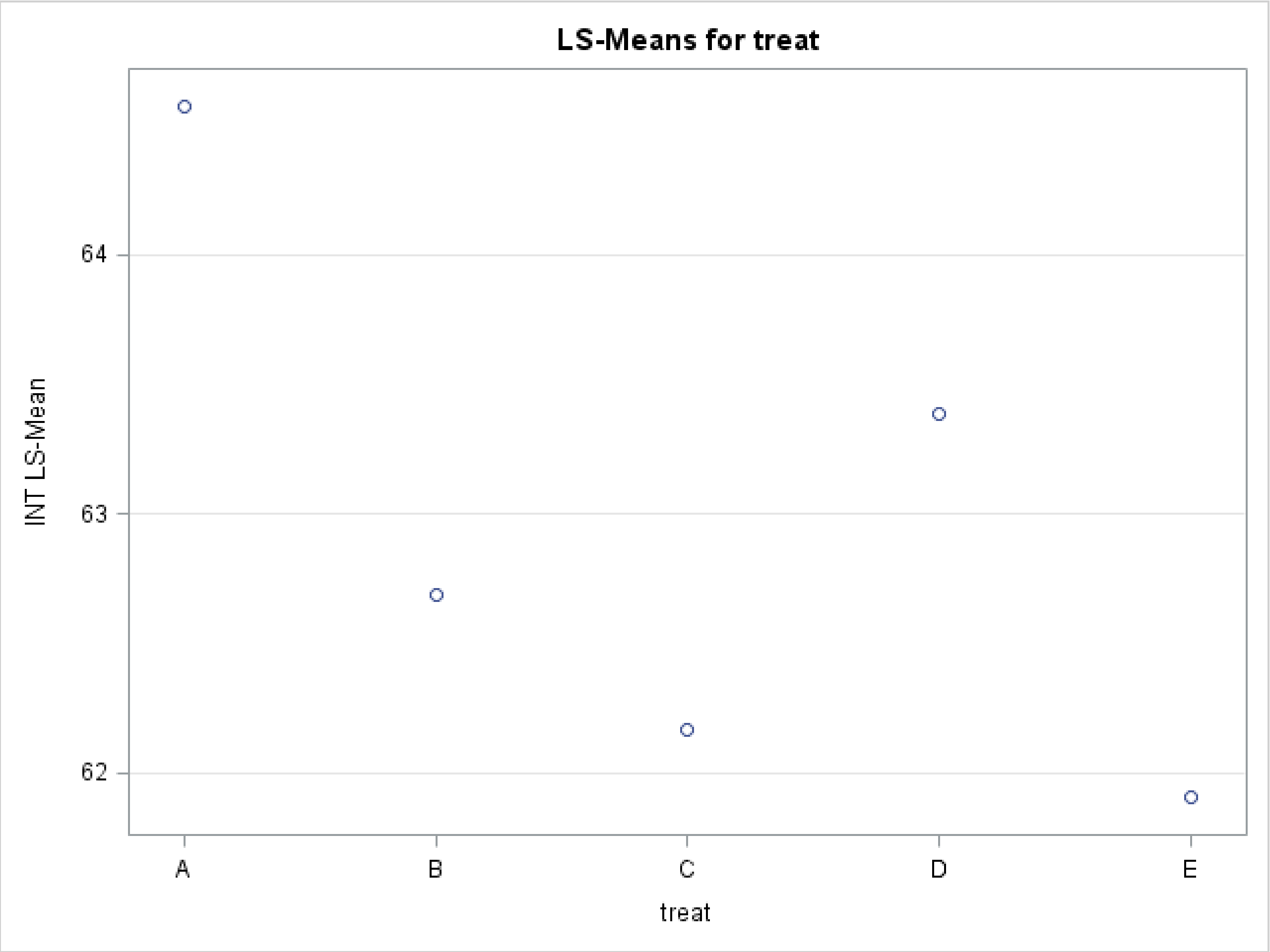
treat	INT LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	64.5740000	1.5603347	<.0001	1
B	62.6900000	1.5603347	<.0001	2
C	62.1660000	1.5603347	<.0001	3
D	63.3820000	1.5603347	<.0001	4
E	61.9060000	1.5603347	<.0001	5

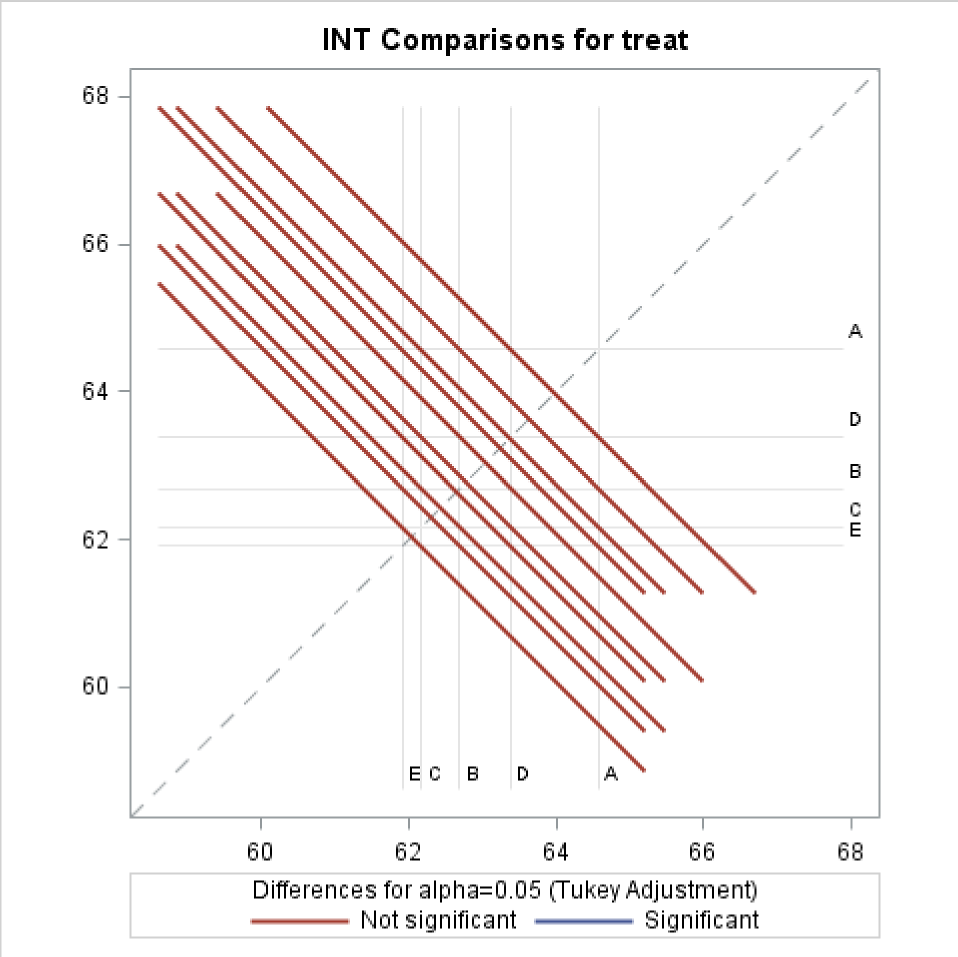
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: INT

i/j	1	2	3	4	5
1		0.9102	0.8089	0.9819	0.7463
2	0.9102		0.9992	0.9977	0.9963
3	0.8089	0.9992		0.9805	1.0000
4	0.9819	0.9977	0.9805		0.9609

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: INT

i/j	1	2	3	4	5
5	0.7463	0.9963	1.0000	0.9609	





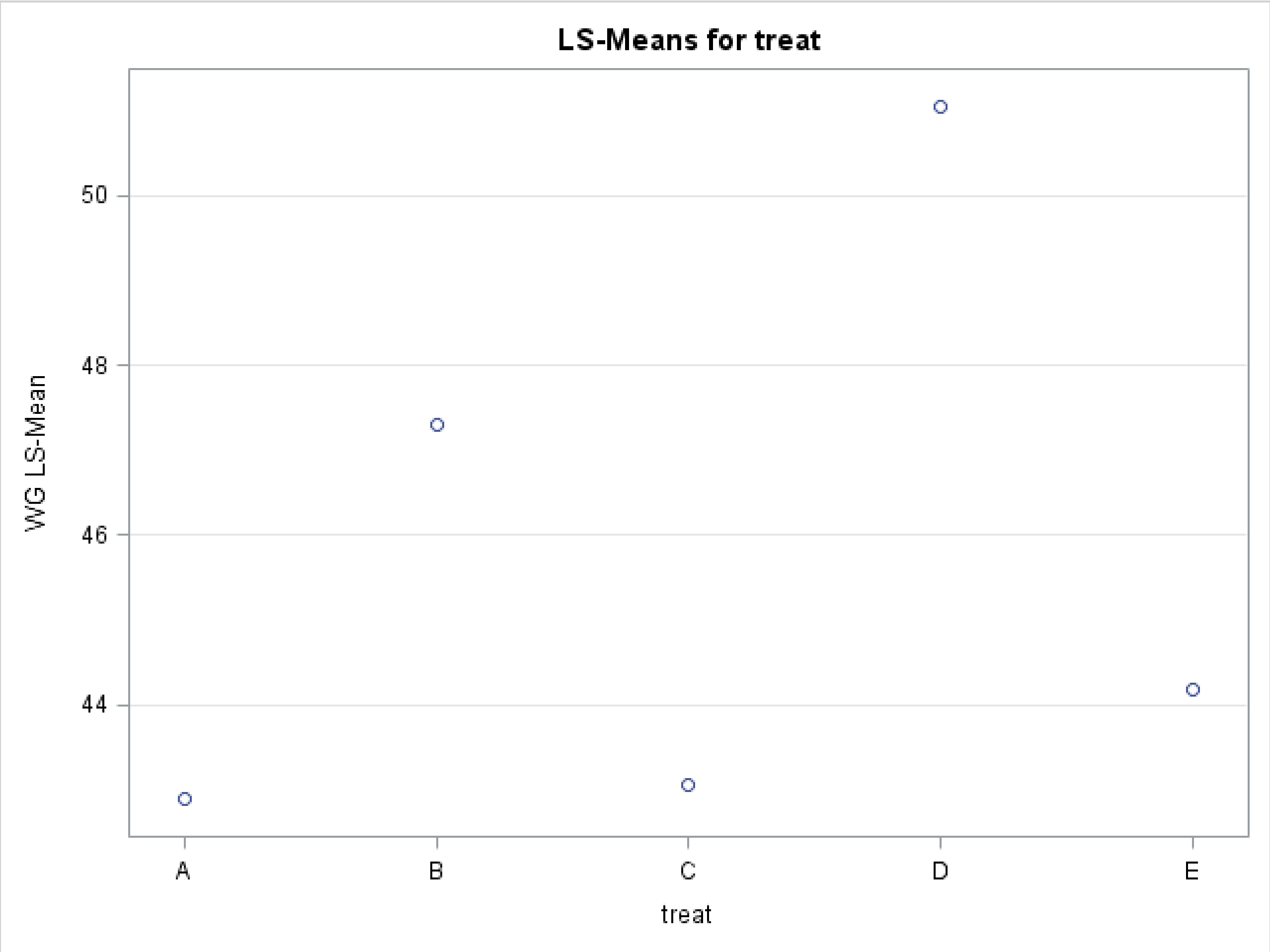
treat	WG LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	42.8860000	5.6580805	<.0001	1
B	47.3000000	5.6580805	<.0001	2
C	43.0620000	5.6580805	<.0001	3
D	51.0560000	5.6580805	<.0001	4
E	44.1860000	5.6580805	<.0001	5

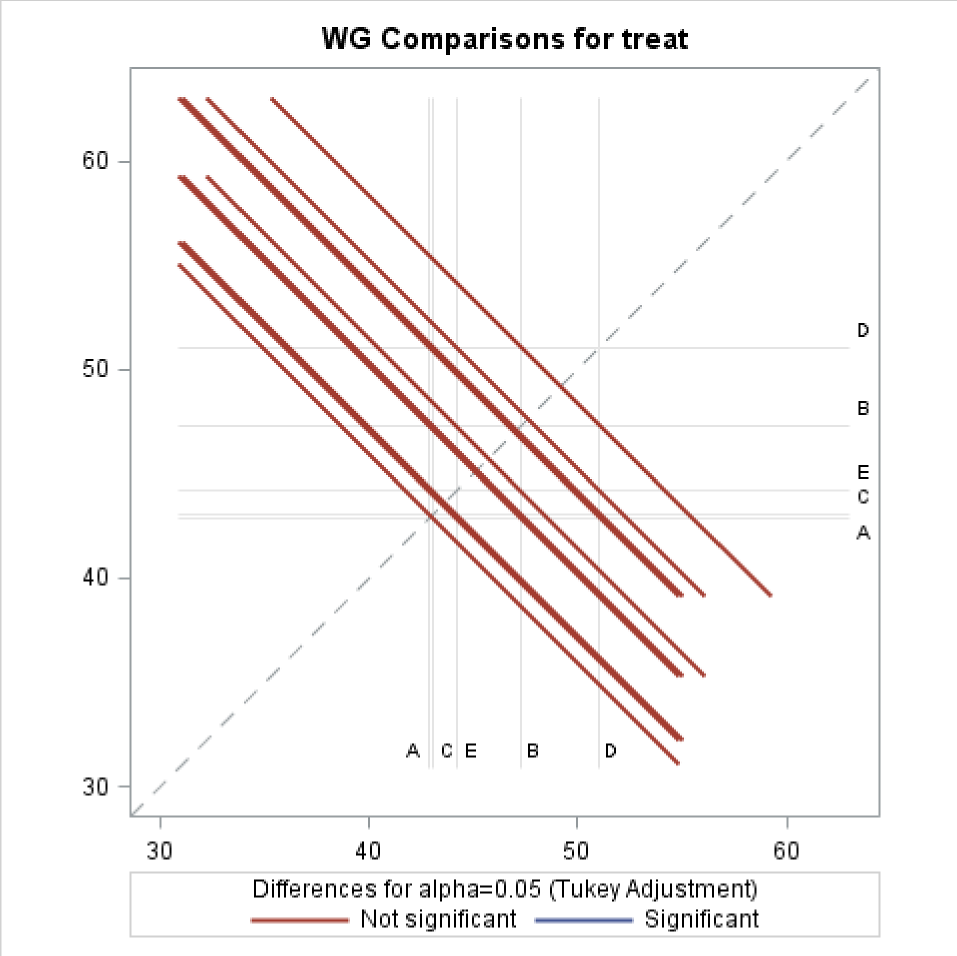
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: WG

i/j	1	2	3	4	5
1		0.9805	1.0000	0.8427	0.9998
2	0.9805		0.9832	0.9893	0.9947
3	1.0000	0.9832		0.8527	0.9999
4	0.8427	0.9893	0.8527		0.9085

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: WG

i/j	1	2	3	4	5
5	0.9998	0.9947	0.9999	0.9085	





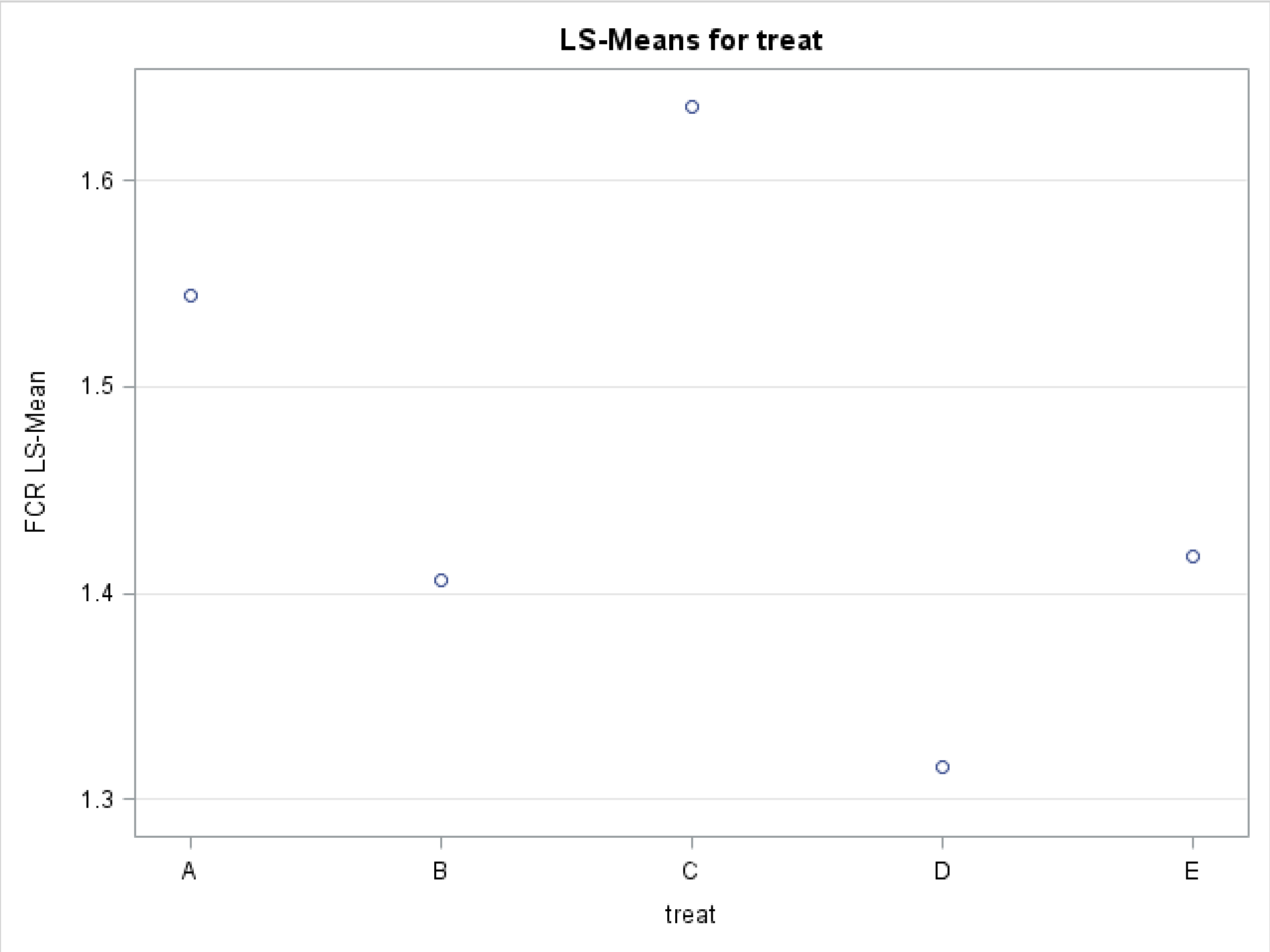
treat	FCR LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	1.54400000	0.19198333	<.0001	1
B	1.40600000	0.19198333	<.0001	2
C	1.63600000	0.19198333	<.0001	3
D	1.31600000	0.19198333	<.0001	4
E	1.41800000	0.19198333	<.0001	5

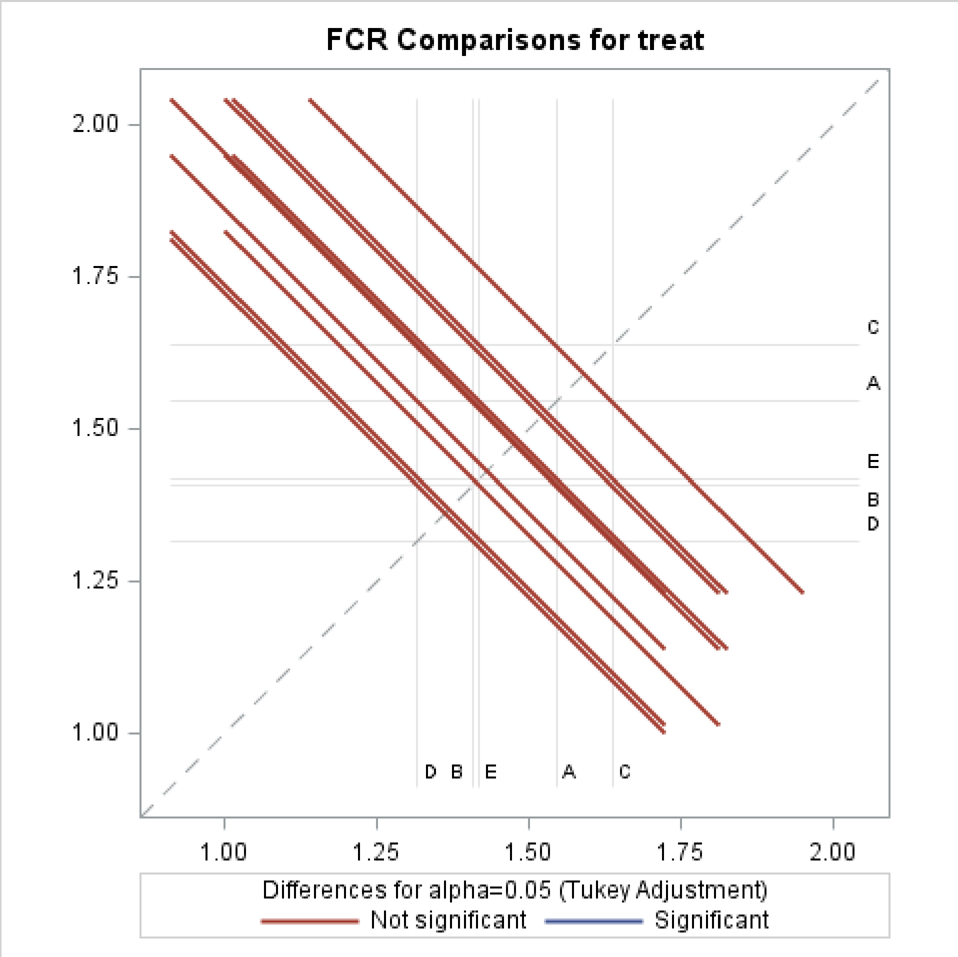
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: FCR

i/j	1	2	3	4	5
1		0.9856	0.9969	0.9149	0.9897
2	0.9856		0.9124	0.9972	1.0000
3	0.9969	0.9124		0.7631	0.9267
4	0.9149	0.9972	0.7631		0.9954

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: FCR

i/j	**1**	**2**	**3**	**4**	**5**
5	0.9897	1.0000	0.9267	0.9954	





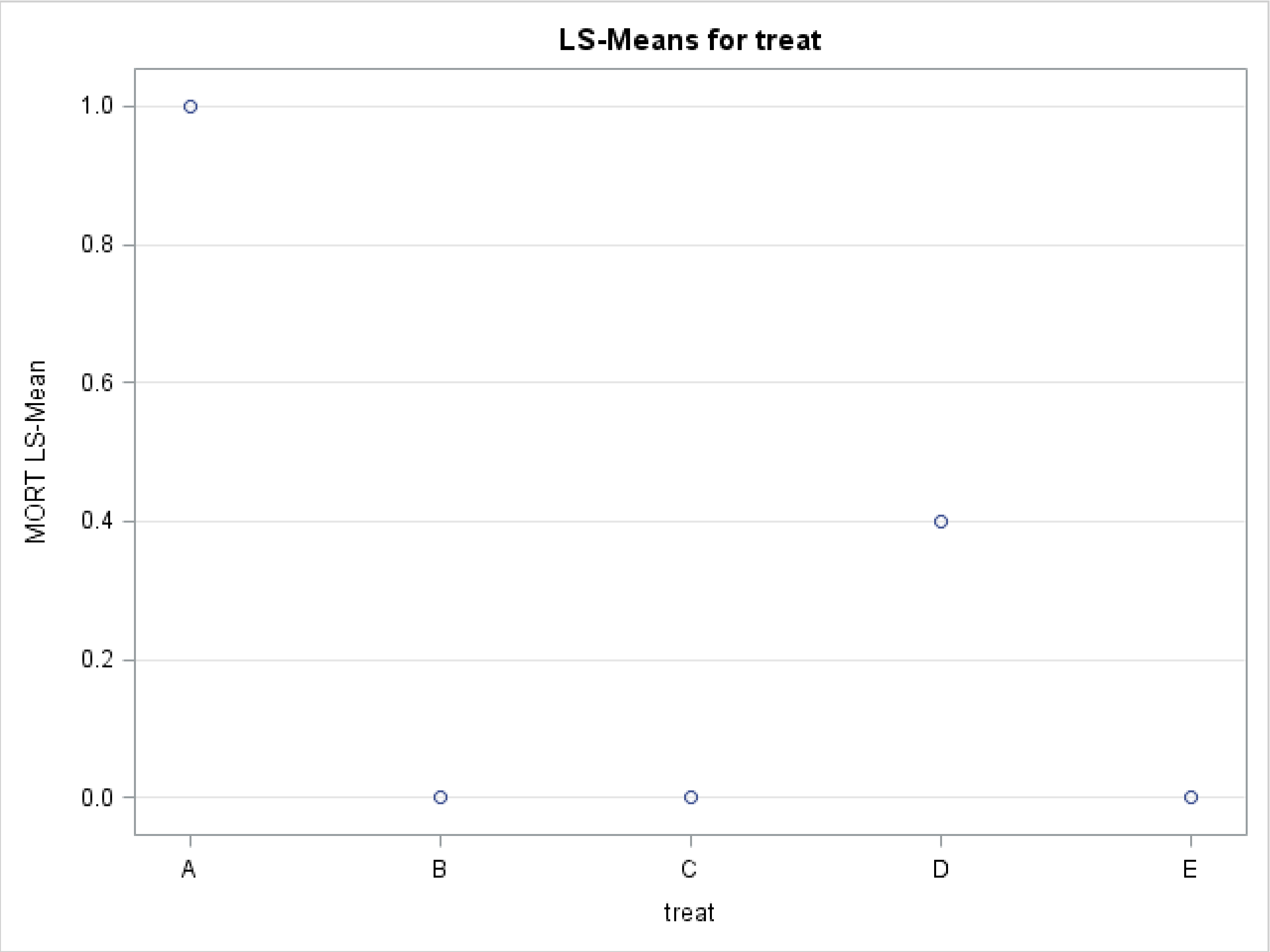
treat	MORT LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	1.00000000	0.22803509	0.0003	1
B	0.00000000	0.22803509	1.0000	2
C	0.00000000	0.22803509	1.0000	3
D	0.40000000	0.22803509	0.0947	4
E	0.00000000	0.22803509	1.0000	5

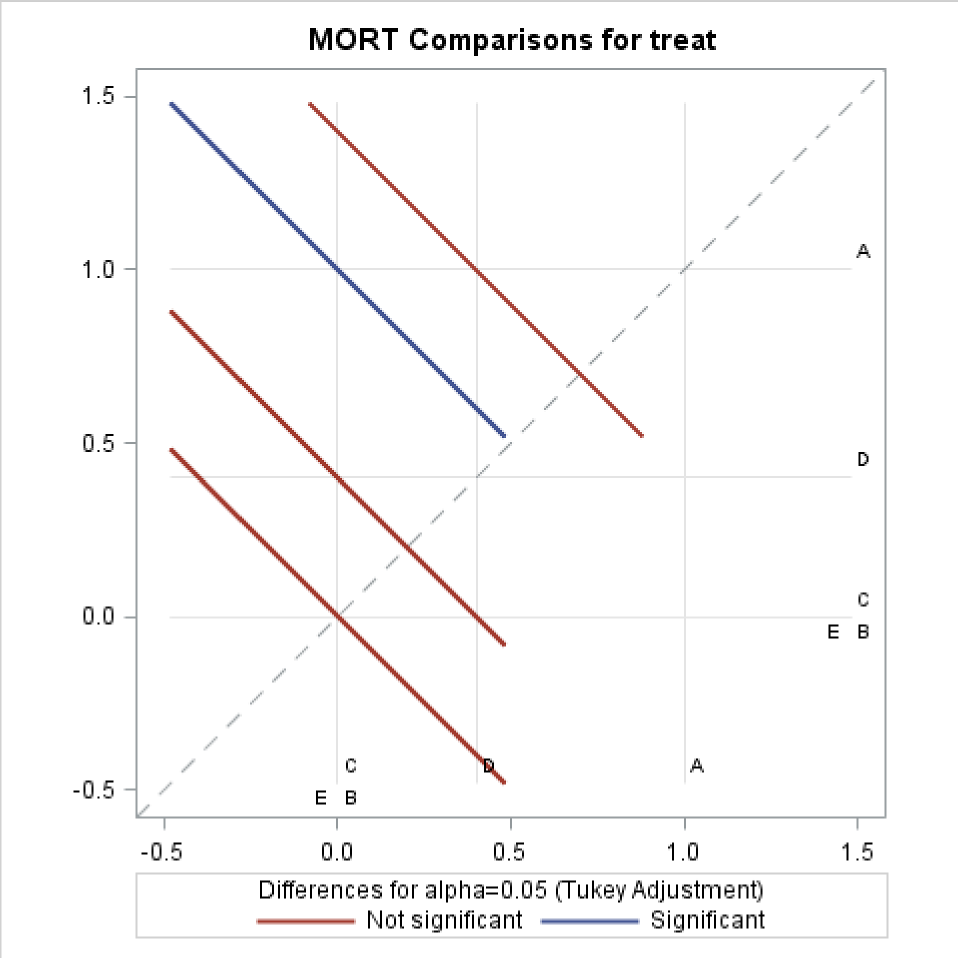
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: MORT

i/j	1	2	3	4	5
1		0.0400	0.0400	0.3691	0.0400
2	0.0400		1.0000	0.7287	1.0000
3	0.0400	1.0000		0.7287	1.0000
4	0.3691	0.7287	0.7287		0.7287

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: MORT

i/j	1	2	3	4	5
5	0.0400	1.0000	1.0000	0.7287	





The SAS System

The GLM Procedure

Class Level Information

Class	Levels	Values
treat	5	A B C D E

Number of Observations Read	25
Number of Observations Used	25

The GLM Procedure

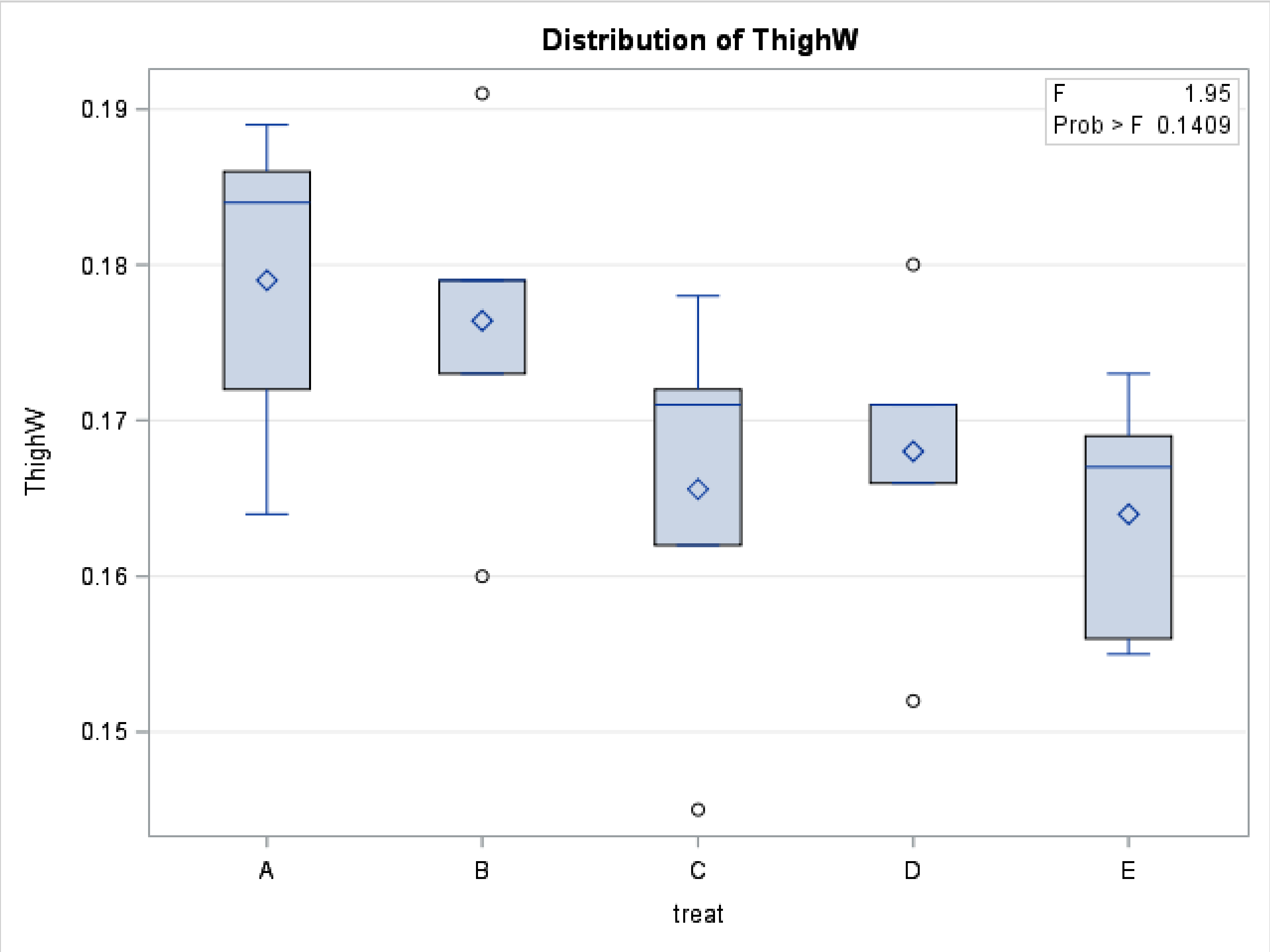
Dependent Variable: ThighW

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.00089760	0.00022440	1.95	0.1409
Error	20	0.00229840	0.00011492		
Corrected Total	24	0.00319600			

R-Square	Coeff Var	Root MSE	ThighW Mean
0.280851	6.283748	0.010720	0.170600

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	0.00089760	0.00022440	1.95	0.1409

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	0.00089760	0.00022440	1.95	0.1409



The SAS System

The GLM Procedure

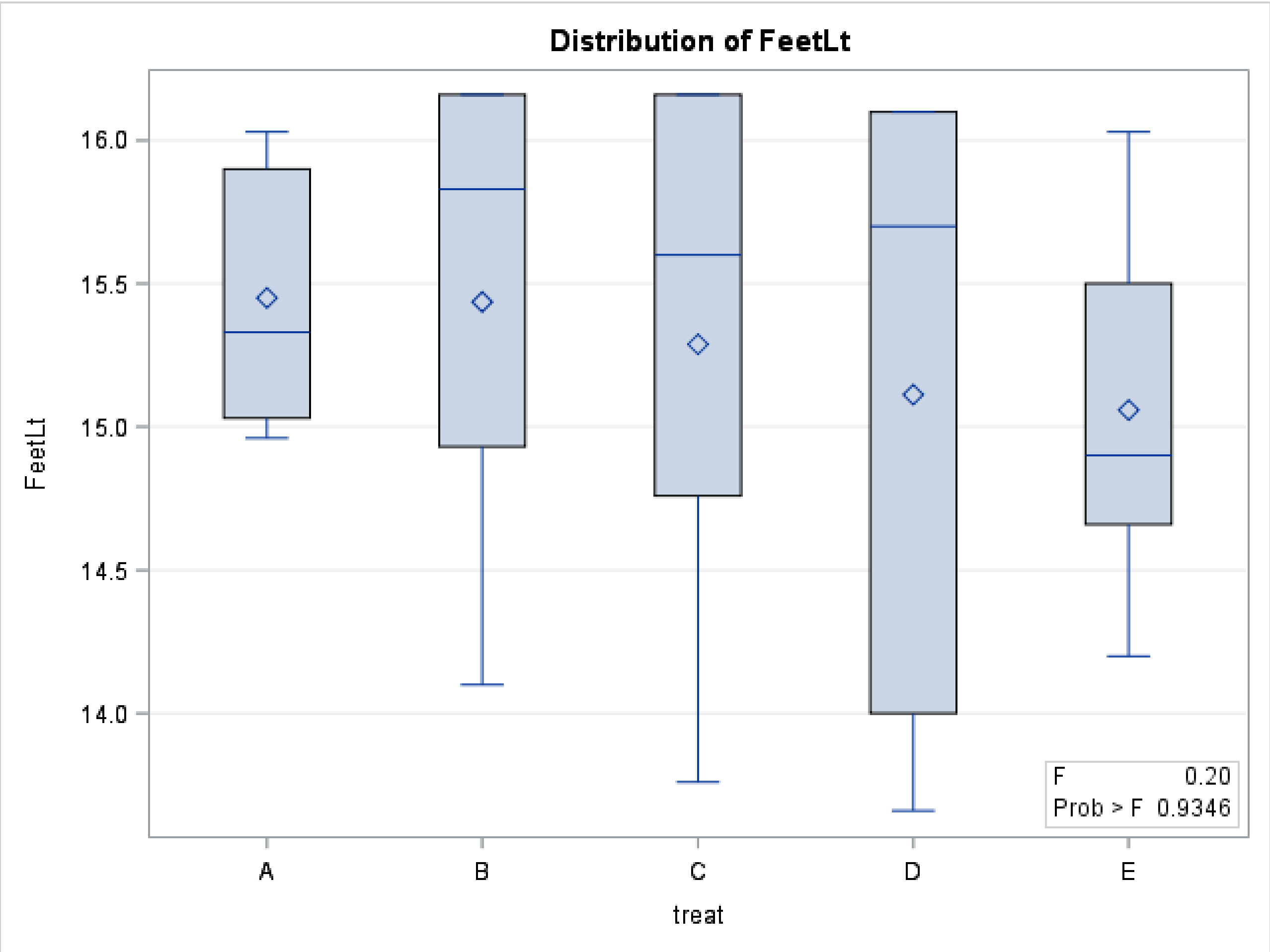
Dependent Variable: FeetLt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.65090400	0.16272600	0.20	0.9346
Error	20	16.14856000	0.80742800		
Corrected Total	24	16.79946400			

R-Square	Coeff Var	Root MSE	FeetLt Mean
0.038746	5.885007	0.898570	15.26880

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	0.65090400	0.16272600	0.20	0.9346

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	0.65090400	0.16272600	0.20	0.9346



The SAS System

The GLM Procedure

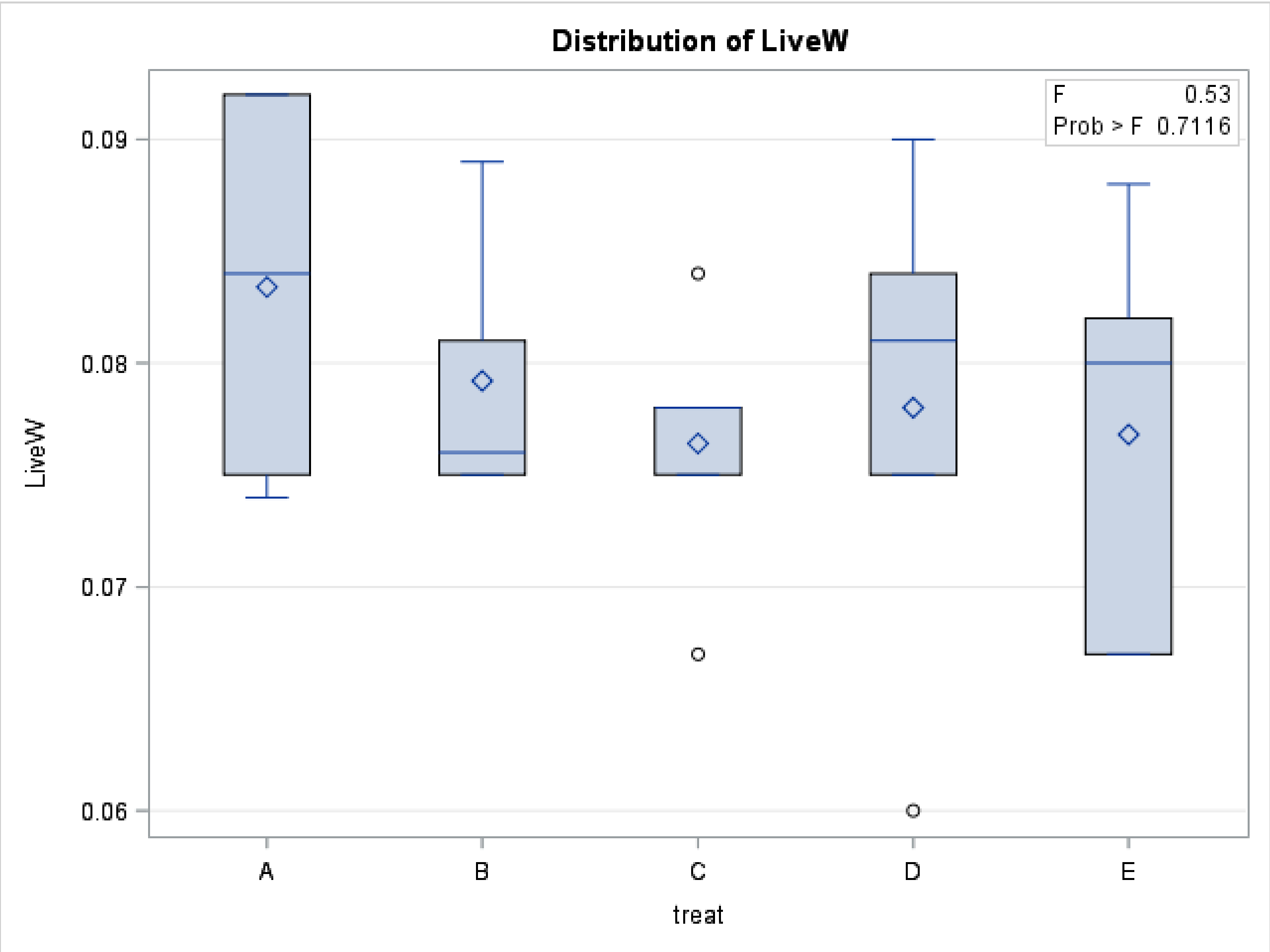
Dependent Variable: LiveW

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.00015856	0.00003964	0.53	0.7116
Error	20	0.00148200	0.00007410		
Corrected Total	24	0.00164056			

R-Square	Coeff Var	Root MSE	LiveW Mean
0.096650	10.92958	0.008608	0.078760

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	0.00015856	0.00003964	0.53	0.7116

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	0.00015856	0.00003964	0.53	0.7116



The SAS System

The GLM Procedure

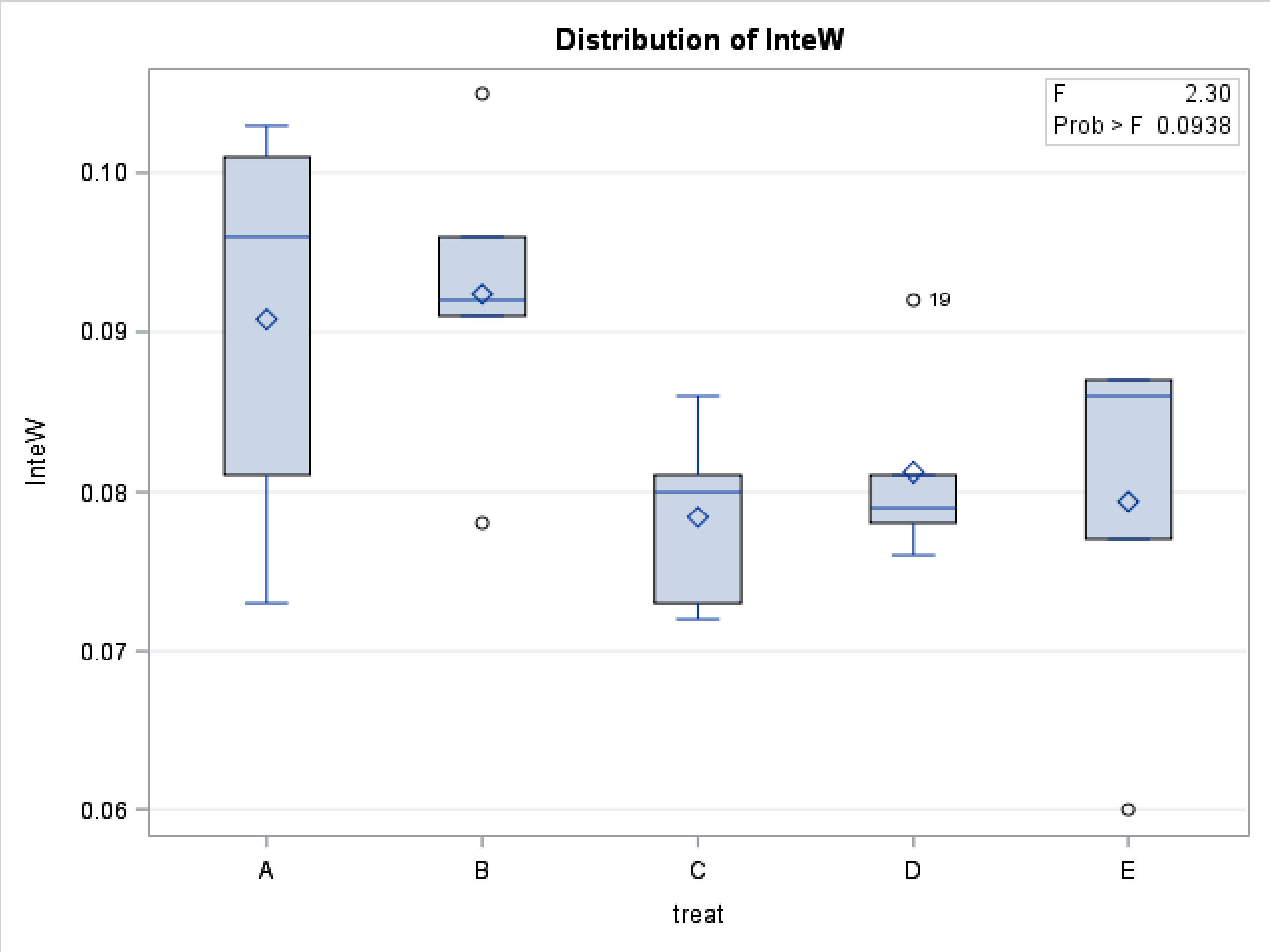
Dependent Variable: InteW

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.00088096	0.00022024	2.30	0.0938
Error	20	0.00191120	0.00009556		
Corrected Total	24	0.00279216			

R-Square	Coeff Var	Root MSE	InteW Mean
0.315512	11.57684	0.009775	0.084440

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	0.00088096	0.00022024	2.30	0.0938

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	0.00088096	0.00022024	2.30	0.0938



The SAS System

The GLM Procedure

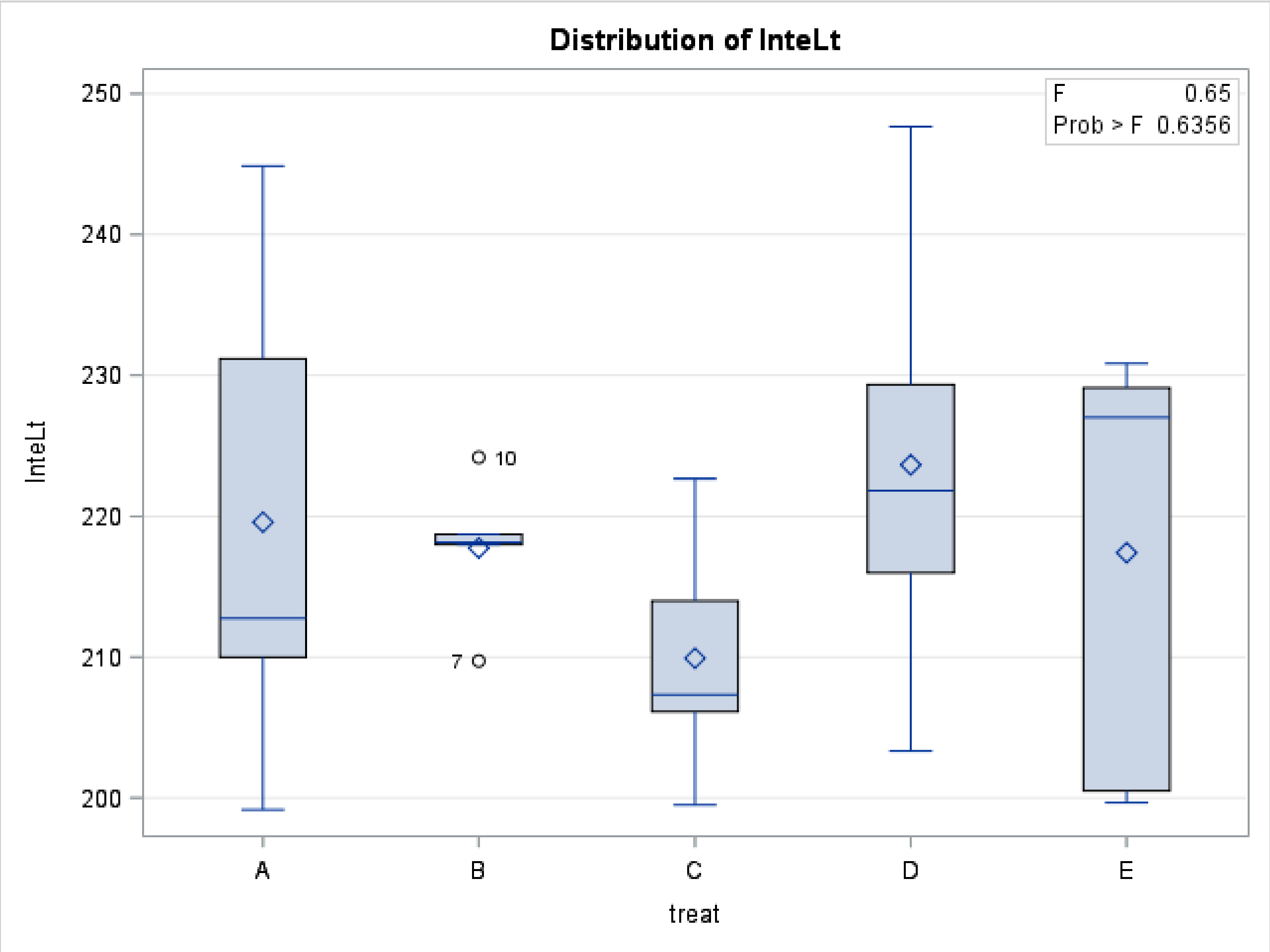
Dependent Variable: InteLt

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	496.357544	124.089386	0.65	0.6356
Error	20	3836.850200	191.842510		
Corrected Total	24	4333.207744			

R-Square	Coeff Var	Root MSE	InteLt Mean
0.114547	6.363373	13.85072	217.6632

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	496.3575440	124.0893860	0.65	0.6356

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	496.3575440	124.0893860	0.65	0.6356



The GLM Procedure

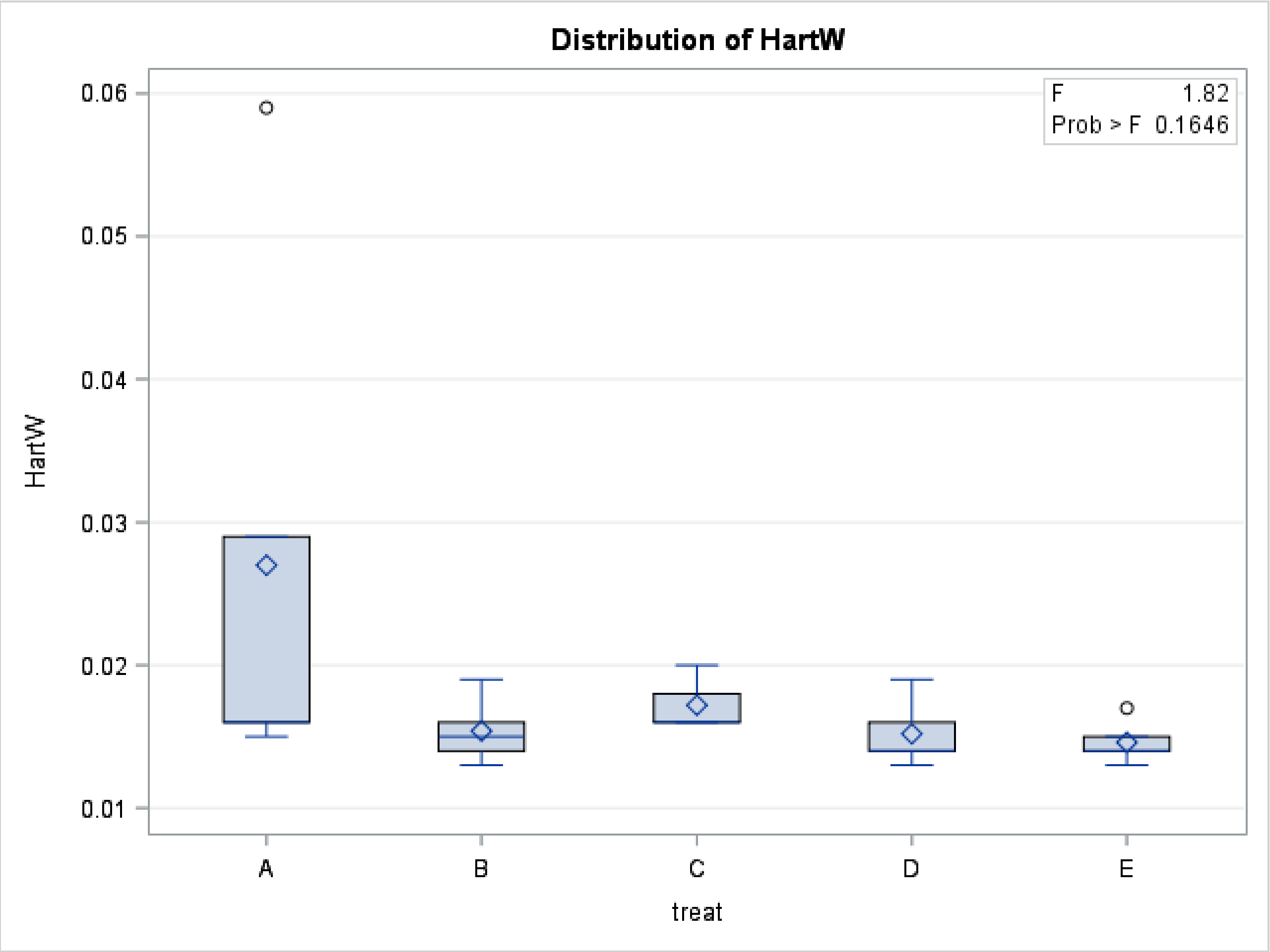
Dependent Variable: HartW

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.00053864	0.00013466	1.82	0.1646
Error	20	0.00148000	0.00007400		
Corrected Total	24	0.00201864			

R-Square	Coeff Var	Root MSE	HartW Mean
0.266833	48.11144	0.008602	0.017880

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	0.00053864	0.00013466	1.82	0.1646

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	0.00053864	0.00013466	1.82	0.1646



The GLM Procedure

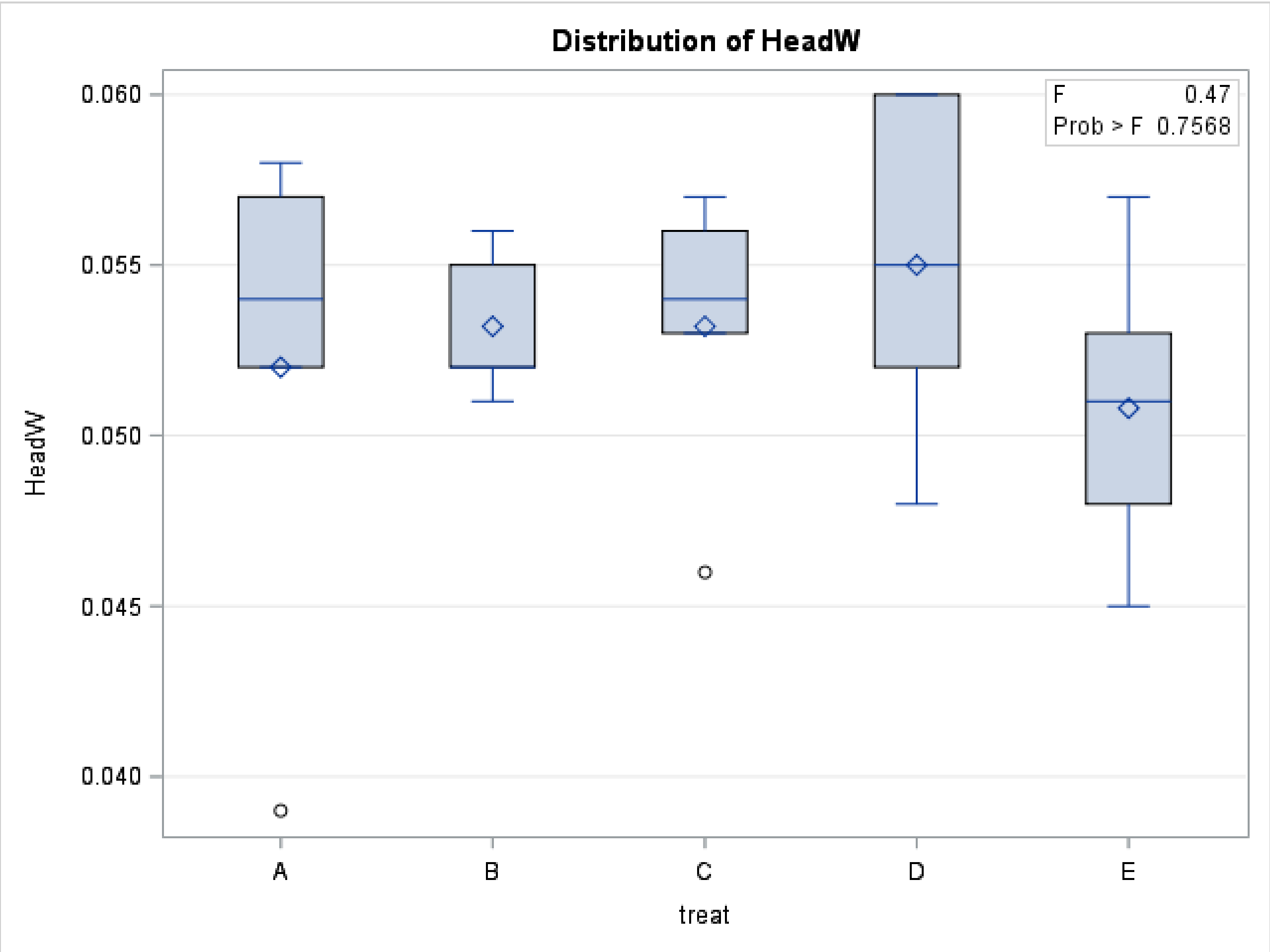
Dependent Variable: HeadW

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.00004896	0.00001224	0.47	0.7568
Error	20	0.00052040	0.00002602		
Corrected Total	24	0.00056936			

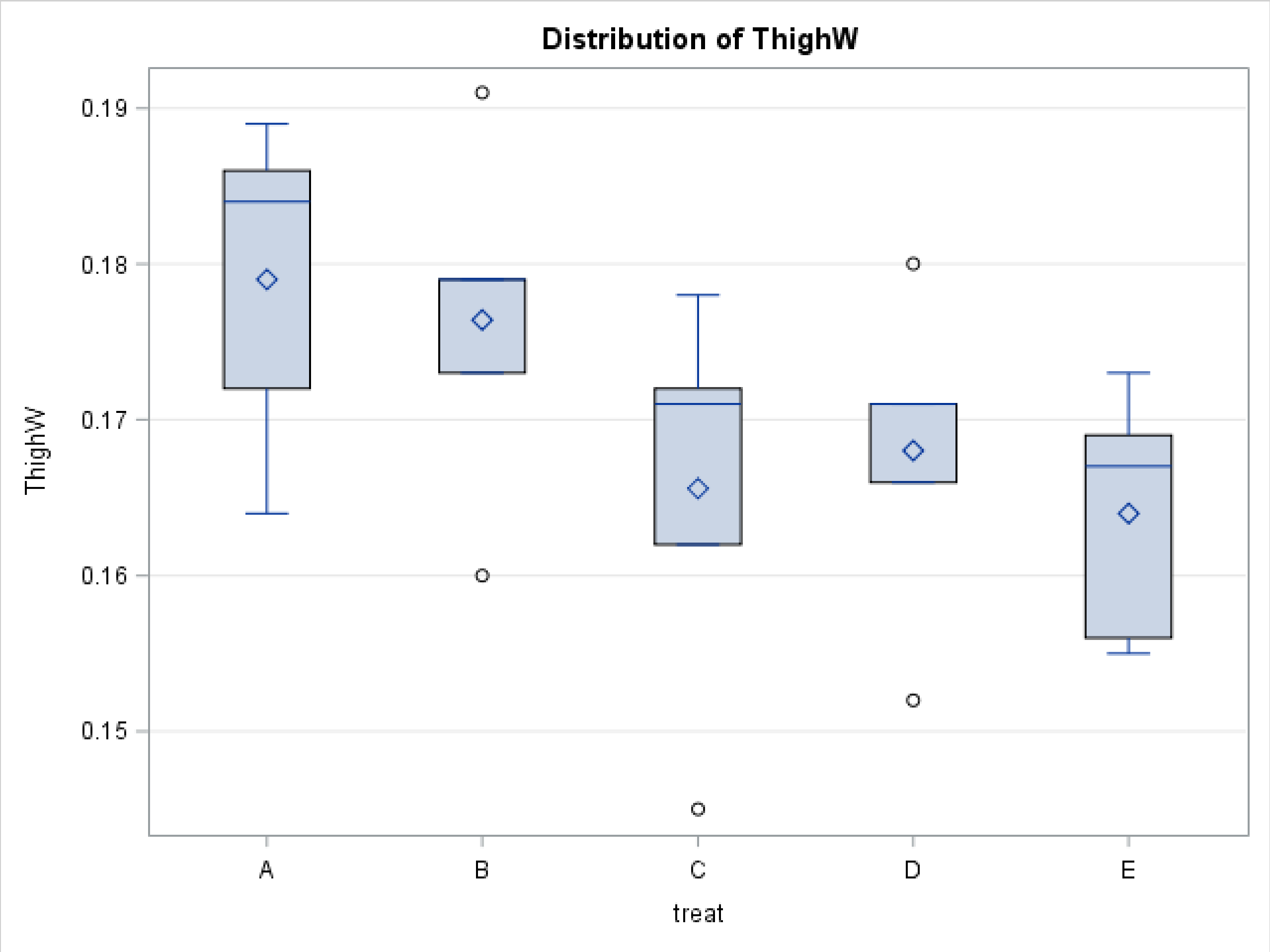
R-Square	Coeff Var	Root MSE	HeadW Mean
0.085991	9.653634	0.005101	0.052840

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	0.00004896	0.00001224	0.47	0.7568

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	0.00004896	0.00001224	0.47	0.7568



The GLM Procedure



The GLM Procedure

t Tests (LSD) for ThighW

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.000115
Critical Value of t	2.08596
Least Significant Difference	0.0141

Means with the same letter are not significantly different.

t Grouping		Mean	N	treat
	A	0.179000	5	A
	A			
B	A	0.176400	5	B
B	A			
B	A	0.168000	5	D
B	A			
B	A	0.165600	5	C
B				
B		0.164000	5	E

The GLM Procedure

Duncan's Multiple Range Test for ThighW

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.000115

Number of Means	**2**	**3**	**4**	**5**
Critical Range	.01414	.01485	.01529	.01560

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treat
A	0.179000	5	A
A			
A	0.176400	5	B
A			
A	0.168000	5	D
A			
A	0.165600	5	C
A			
A	0.164000	5	E

The GLM Procedure

Tukey's Studentized Range (HSD) Test for ThighW

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

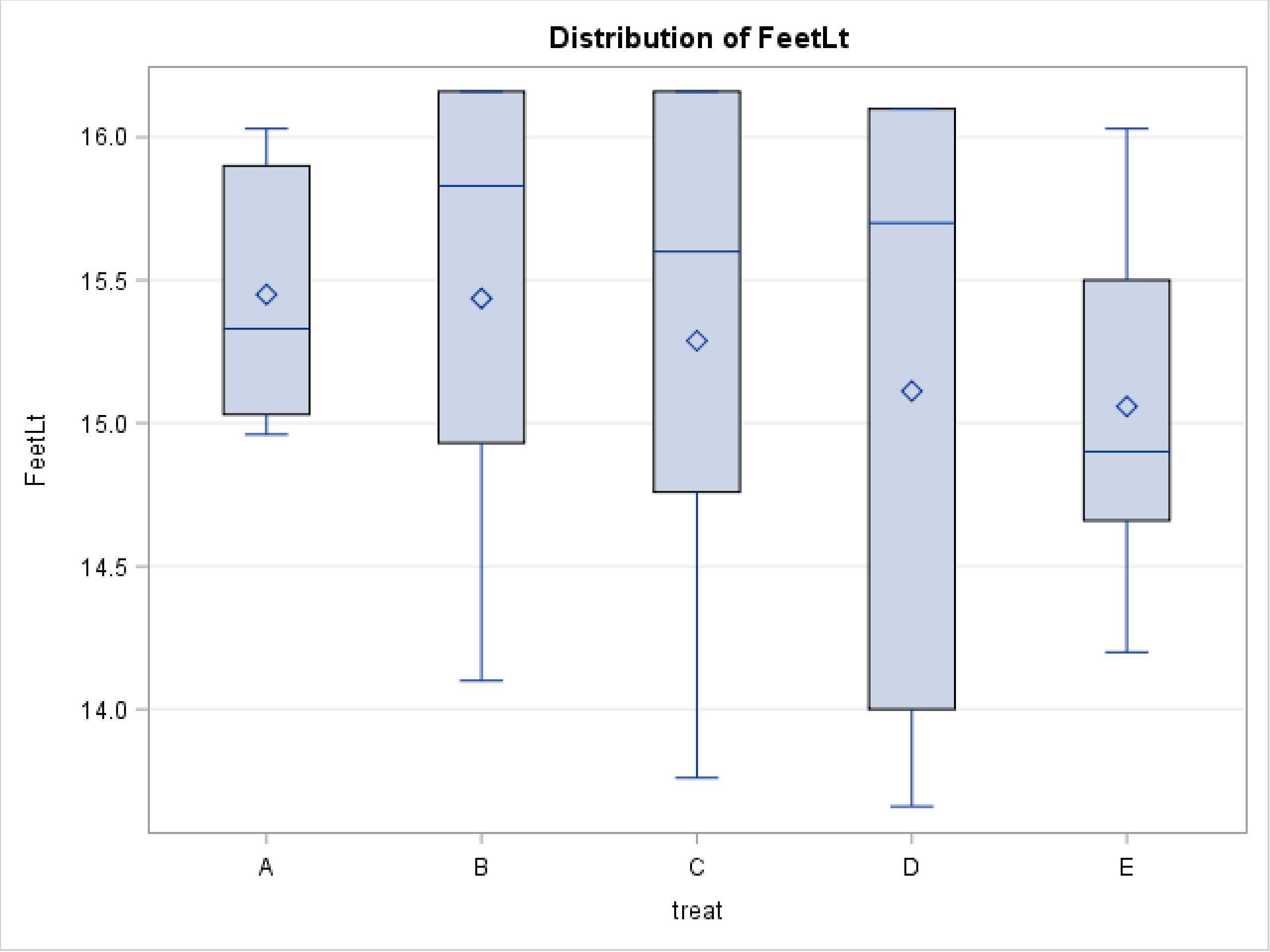
Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.000115
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.0203

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	0.179000	5	A
A			
A	0.176400	5	B
A			
A	0.168000	5	D
A			
A	0.165600	5	C
A			
A	0.164000	5	E

The SAS System

The GLM Procedure



The GLM Procedure

t Tests (LSD) for FeetLt

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.807428
Critical Value of t	2.08596
Least Significant Difference	1.1855

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	15.4500	5	A
A			
A	15.4360	5	B
A			
A	15.2880	5	C
A			
A	15.1120	5	D
A			
A	15.0580	5	E

The SAS System

The GLM Procedure

Duncan's Multiple Range Test for FeetLt

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.807428

Number of Means	**2**	**3**	**4**	**5**
Critical Range	1.185	1.244	1.282	1.308

Means with the same letter are not significantly different.

Duncan Grouping	**Mean**	**N**	**treat**
A	15.4500	5	A
A			
A	15.4360	5	B
A			
A	15.2880	5	C
A			
A	15.1120	5	D
A			
A	15.0580	5	E

The GLM Procedure

Tukey's Studentized Range (HSD) Test for FeetLt

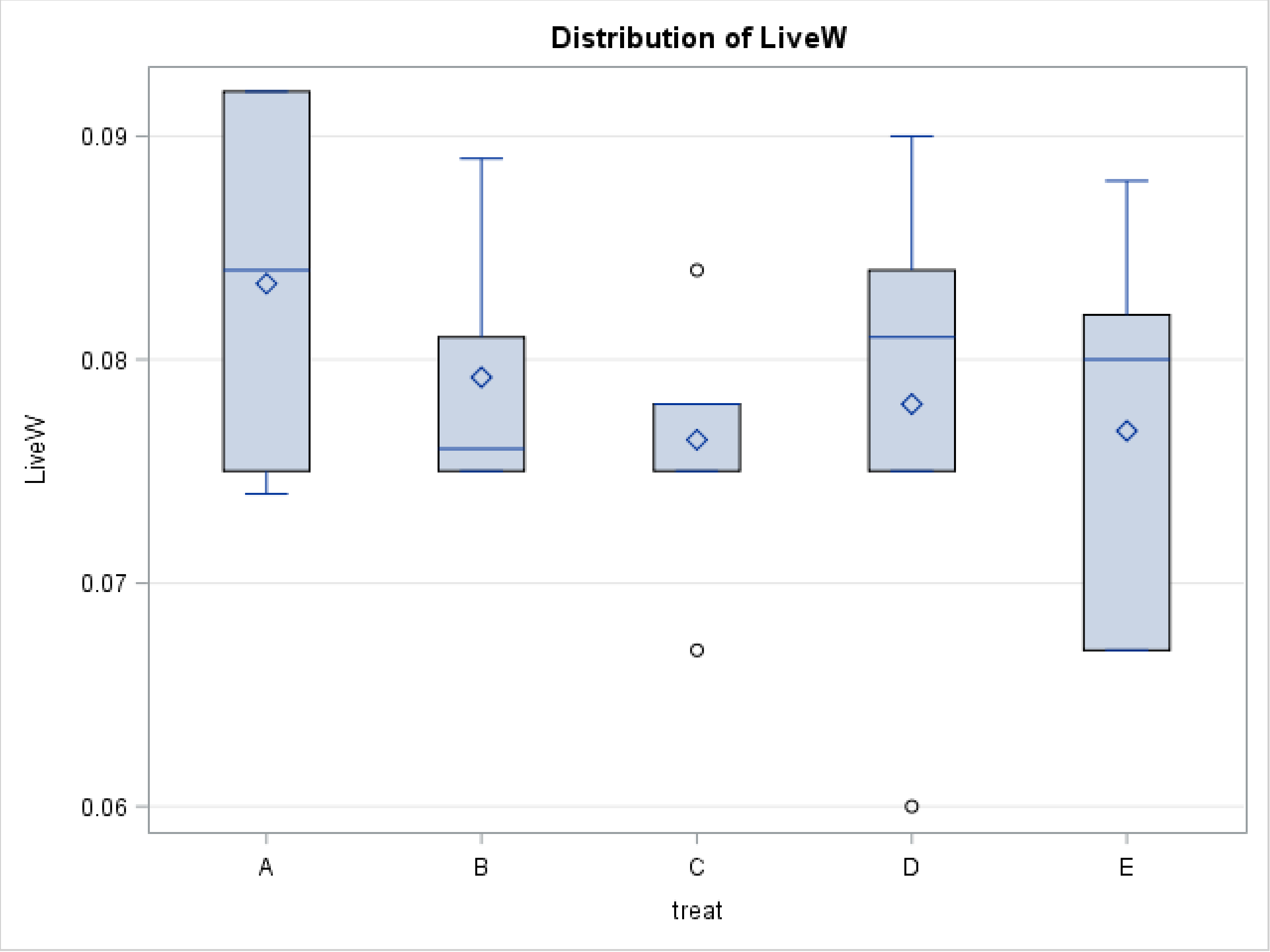
Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.807428
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	1.7006

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	15.4500	5	A
A			
A	15.4360	5	B
A			
A	15.2880	5	C
A			
A	15.1120	5	D
A			
A	15.0580	5	E

The GLM Procedure



The GLM Procedure

t Tests (LSD) for LiveW

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.000074
Critical Value of t	2.08596
Least Significant Difference	0.0114

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	0.083400	5	A
A			
A	0.079200	5	B
A			
A	0.078000	5	D
A			
A	0.076800	5	E
A			
A	0.076400	5	C

The GLM Procedure

Duncan's Multiple Range Test for LiveW

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.000074

Number of Means	**2**	**3**	**4**	**5**
Critical Range	.01136	.01192	.01228	.01253

Means with the same letter are not significantly different.

Duncan Grouping	**Mean**	**N**	**treat**
A	0.083400	5	A
A			
A	0.079200	5	B
A			
A	0.078000	5	D
A			
A	0.076800	5	E
A			
A	0.076400	5	C

The GLM Procedure

Tukey's Studentized Range (HSD) Test for LiveW

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

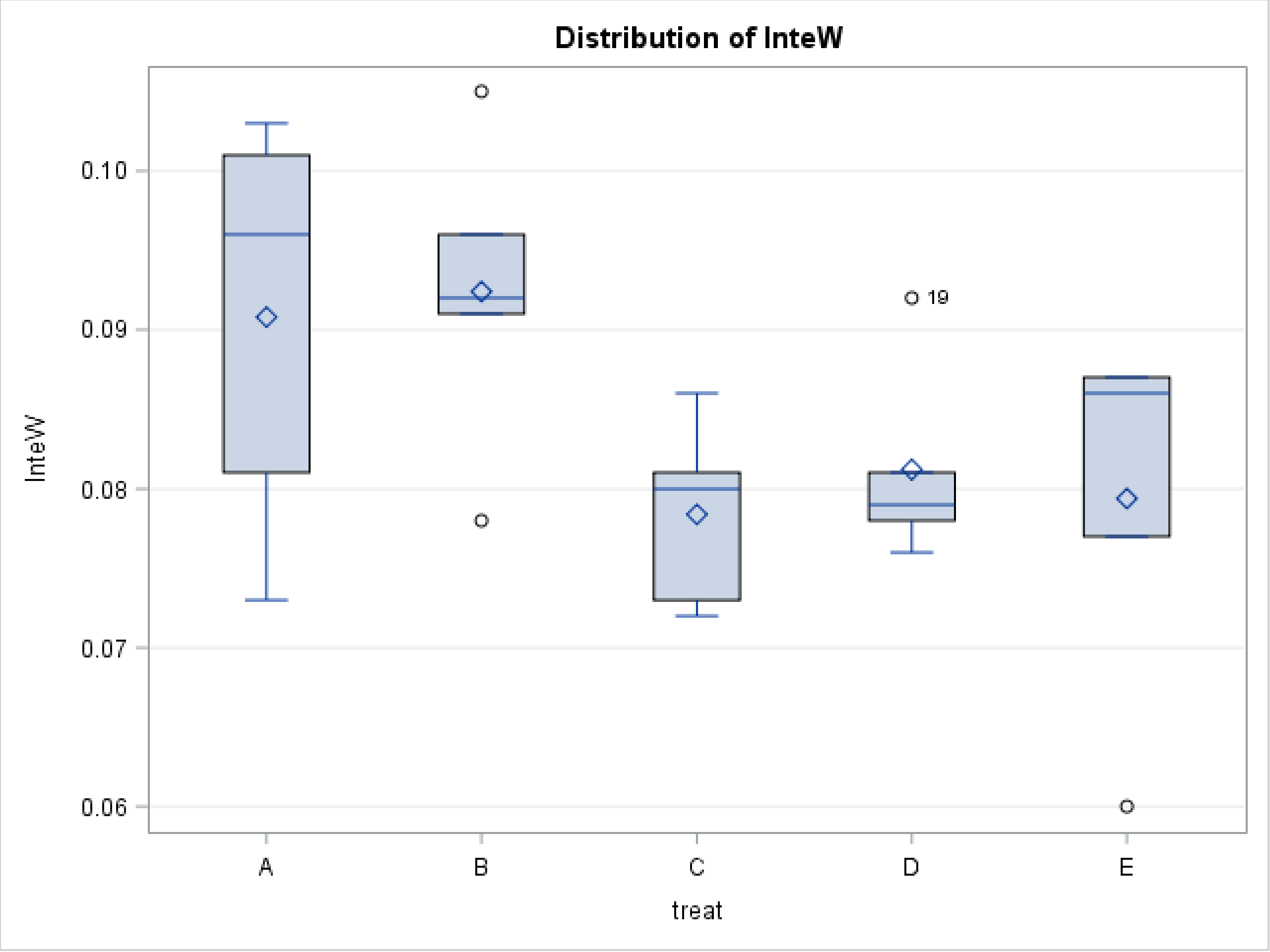
Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.000074
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.0163

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	0.083400	5	A
A			
A	0.079200	5	B
A			
A	0.078000	5	D
A			
A	0.076800	5	E
A			
A	0.076400	5	C

The SAS System

The GLM Procedure



The SAS System

The GLM Procedure

t Tests (LSD) for InteW

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.000096
Critical Value of t	2.08596
Least Significant Difference	0.0129

Means with the same letter are not significantly different.

t Grouping		Mean	N	treat
	A	0.092400	5	B
	A			
B	A	0.090800	5	A
B	A			
B	A	0.081200	5	D
B				
B		0.079400	5	E
B				
B		0.078400	5	C

The GLM Procedure

Duncan's Multiple Range Test for InteW

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.000096

Number of Means	**2**	**3**	**4**	**5**
Critical Range	.01290	.01354	.01394	.01423

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treat
A	0.092400	5	B
A			
A	0.090800	5	A
A			
A	0.081200	5	D
A			
A	0.079400	5	E
A			
A	0.078400	5	C

The GLM Procedure

Tukey's Studentized Range (HSD) Test for InteW

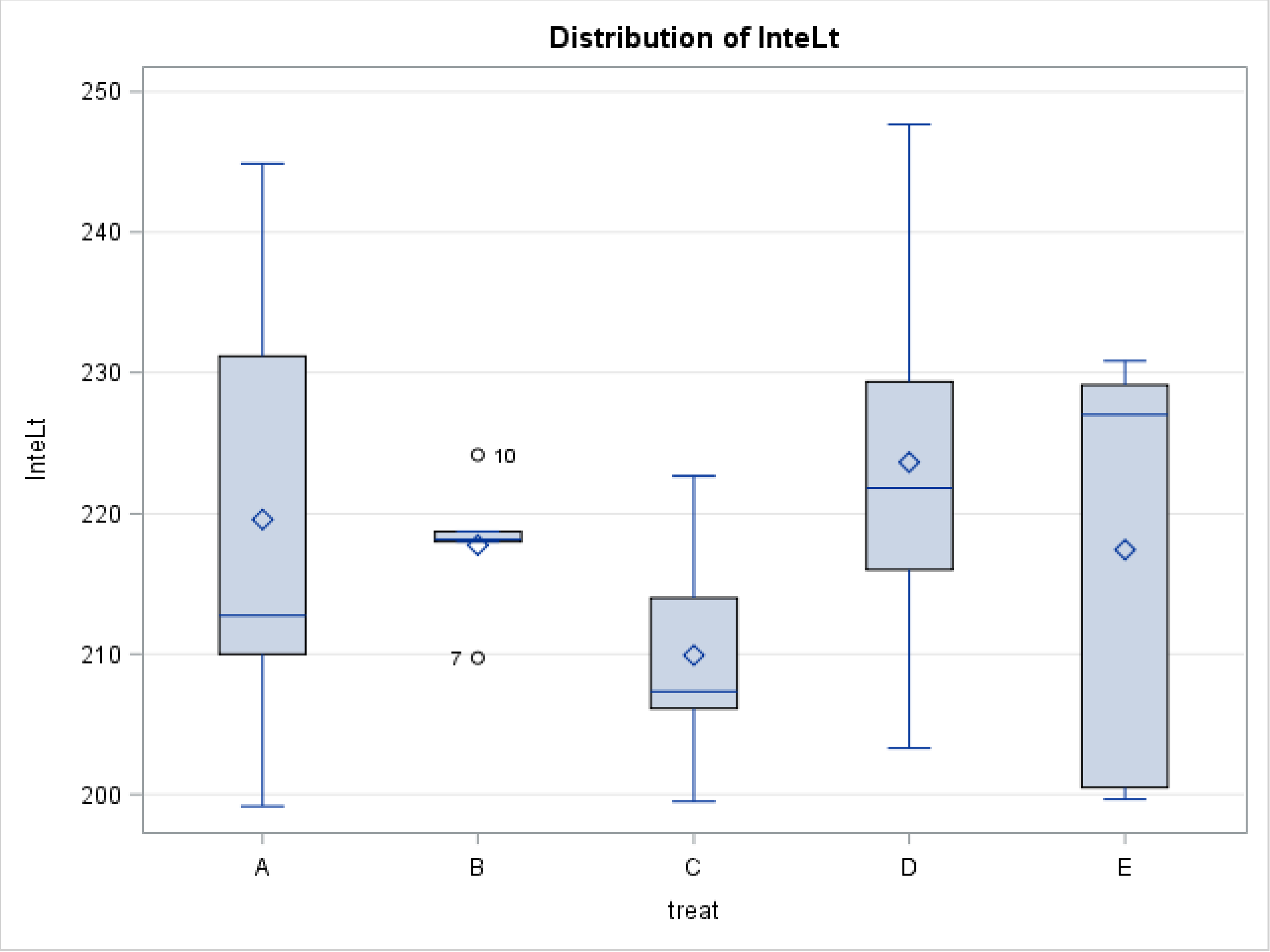
Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.000096
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.0185

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	0.092400	5	B
A			
A	0.090800	5	A
A			
A	0.081200	5	D
A			
A	0.079400	5	E
A			
A	0.078400	5	C

The GLM Procedure



The GLM Procedure

t Tests (LSD) for InteLt

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	191.8425
Critical Value of t	2.08596
Least Significant Difference	18.273

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	223.630	5	D
A			
A	219.588	5	A
A			
A	217.756	5	B
A			
A	217.418	5	E
A			
A	209.924	5	C

The SAS System

The GLM Procedure

Duncan's Multiple Range Test for InteLt

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	191.8425

Number of Means	**2**	**3**	**4**	**5**
Critical Range	18.27	19.18	19.76	20.16

Means with the same letter are not significantly different.

Duncan Grouping	**Mean**	**N**	**treat**
A	223.630	5	D
A			
A	219.588	5	A
A			
A	217.756	5	B
A			
A	217.418	5	E
A			
A	209.924	5	C

The GLM Procedure

Tukey's Studentized Range (HSD) Test for InteLt

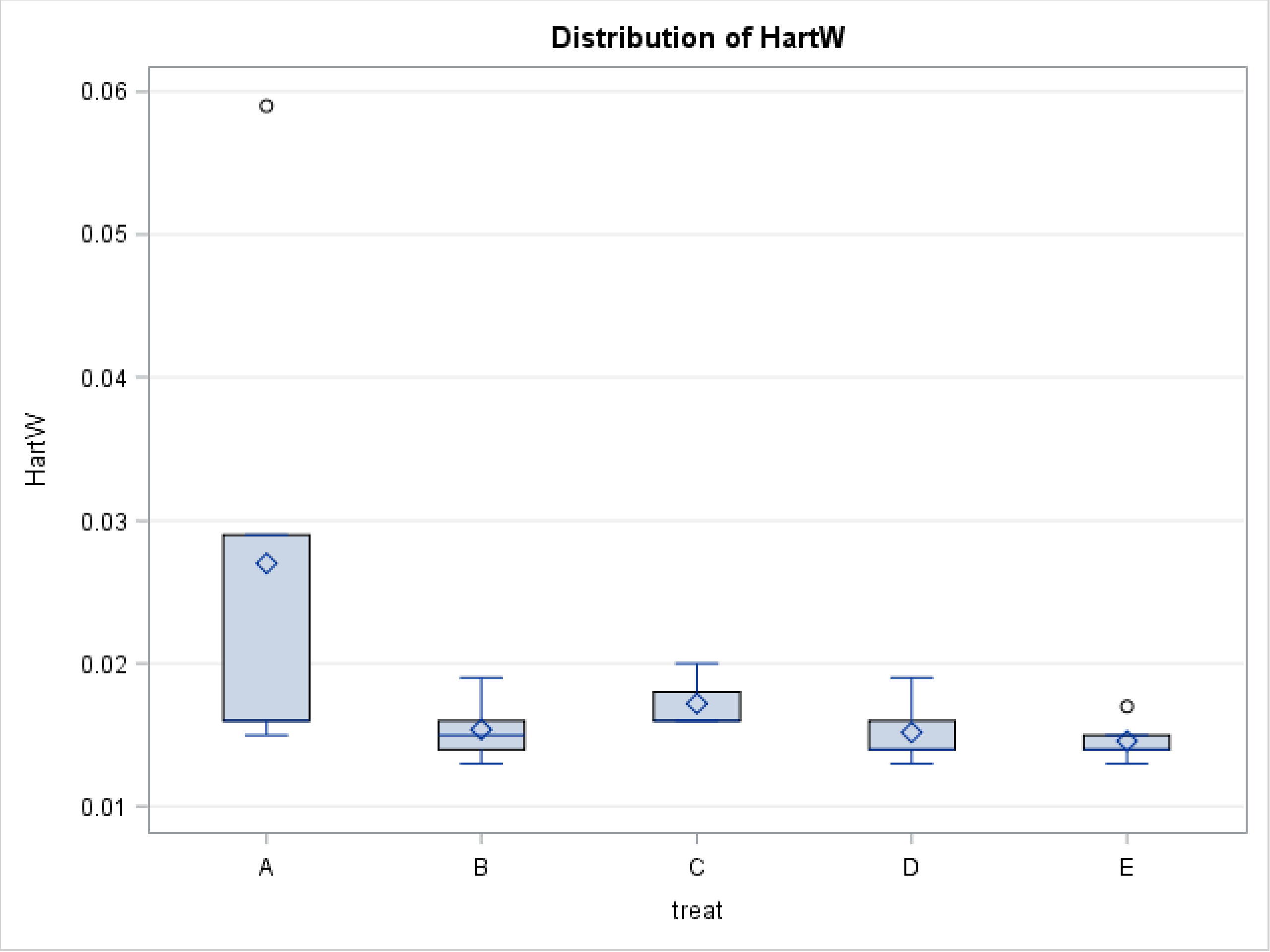
Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	191.8425
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	26.213

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	223.630	5	D
A			
A	219.588	5	A
A			
A	217.756	5	B
A			
A	217.418	5	E
A			
A	209.924	5	C

The GLM Procedure



The GLM Procedure

t Tests (LSD) for HartW

Note: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.000074
Critical Value of t	2.08596
Least Significant Difference	0.0113

Means with the same letter are not significantly different.

t Grouping		Mean	N	treat
	A	0.027000	5	A
	A			
B	A	0.017200	5	C
B				
B		0.015400	5	B
B				
B		0.015200	5	D
B				
B		0.014600	5	E

The GLM Procedure

Duncan's Multiple Range Test for HartW

Note: This test controls the Type I comparison wise error rate, not the experiment wise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.000074

Number of Means	**2**	**3**	**4**	**5**
Critical Range	.01135	.01191	.01227	.01252

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treat
A	0.027000	5	A
A			
A	0.017200	5	C
A			
A	0.015400	5	B
A			
A	0.015200	5	D
A			
A	0.014600	5	E

The GLM Procedure

Tukey's Studentized Range (HSD) Test for HartW

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

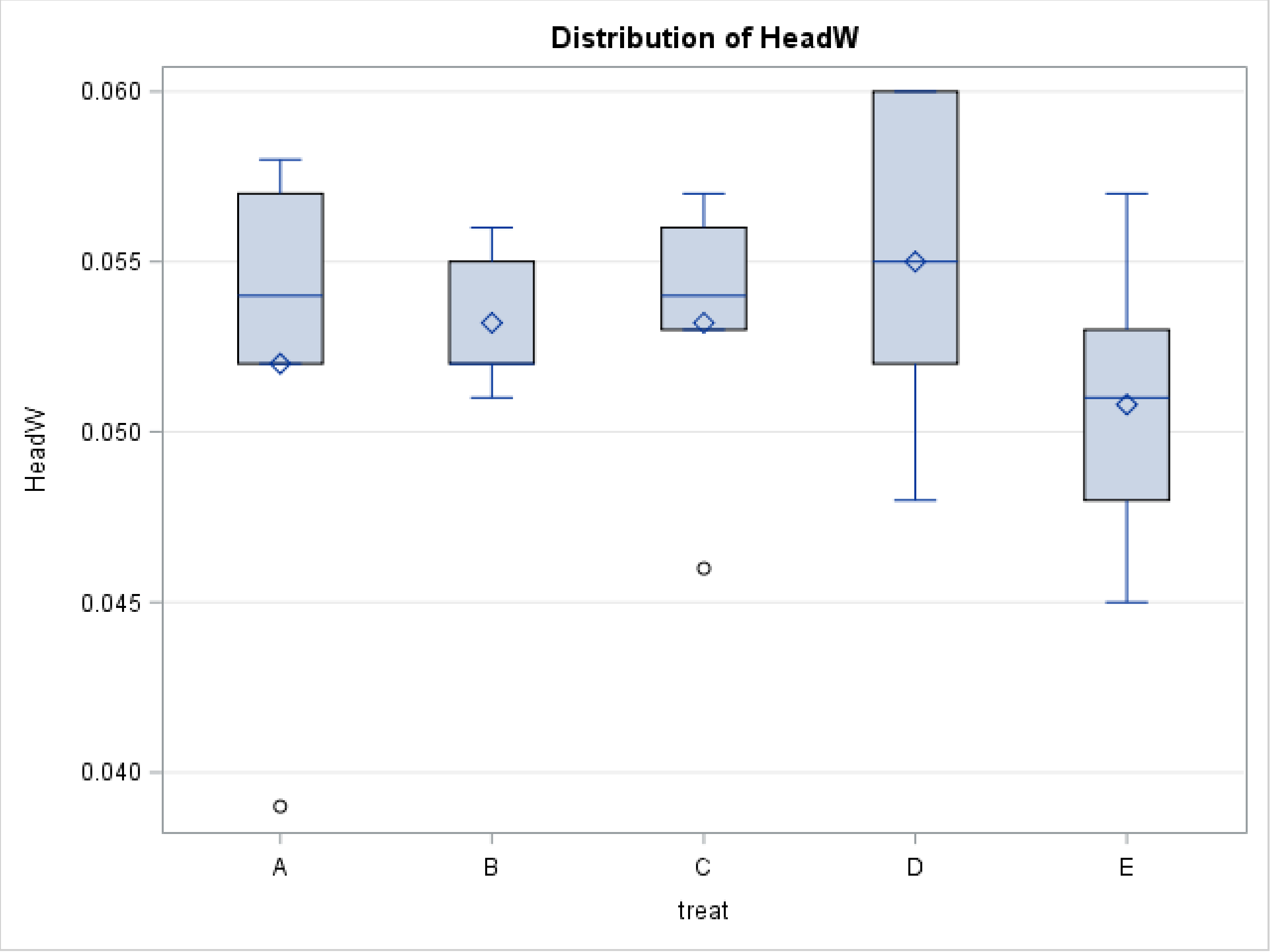
Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.000074
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.0163

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	0.027000	5	A
A			
A	0.017200	5	C
A			
A	0.015400	5	B
A			
A	0.015200	5	D
A			
A	0.014600	5	E

The SAS System

The GLM Procedure



The GLM Procedure

t Tests (LSD) for HeadW

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.000026
Critical Value of t	2.08596
Least Significant Difference	0.0067

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	0.055000	5	D
A			
A	0.053200	5	C
A			
A	0.053200	5	B
A			
A	0.052000	5	A
A			
A	0.050800	5	E

The GLM Procedure

Duncan's Multiple Range Test for HeadW

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.000026

Number of Means	**2**	**3**	**4**	**5**
Critical Range	.006729	.007064	.007276	.007425

Means with the same letter are not significantly different.

Duncan Grouping	**Mean**	**N**	**treat**
A	0.055000	5	D
A			
A	0.053200	5	C
A			
A	0.053200	5	B
A			
A	0.052000	5	A
A			
A	0.050800	5	E

The GLM Procedure

Tukey's Studentized Range (HSD) Test for HeadW

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.000026
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.0097

Means with the same letter are not significantly different.

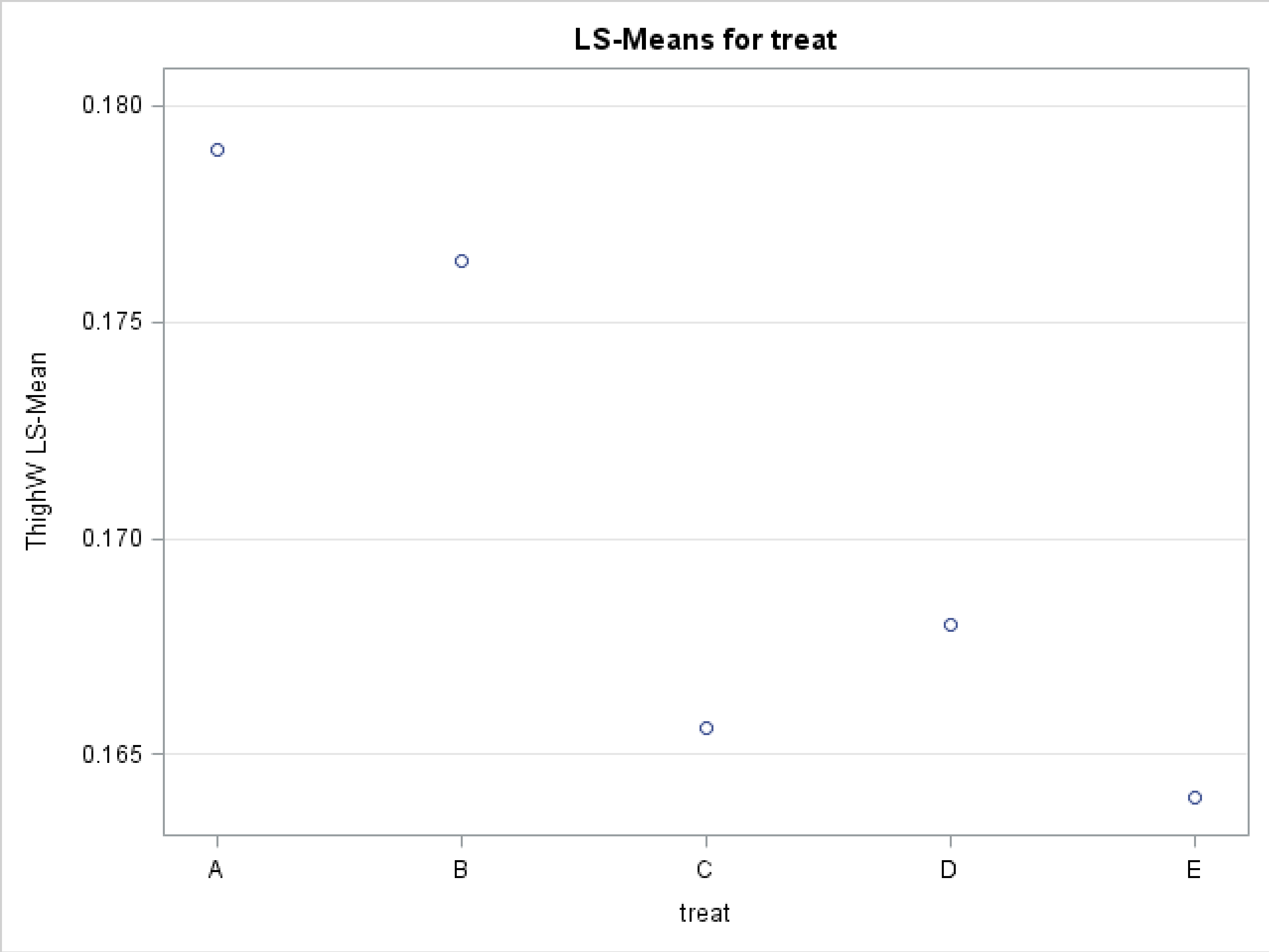
Tukey Grouping	Mean	N	treat
A	0.055000	5	D
A			
A	0.053200	5	C
A			
A	0.053200	5	B
A			
A	0.052000	5	A
A			
A	0.050800	5	E

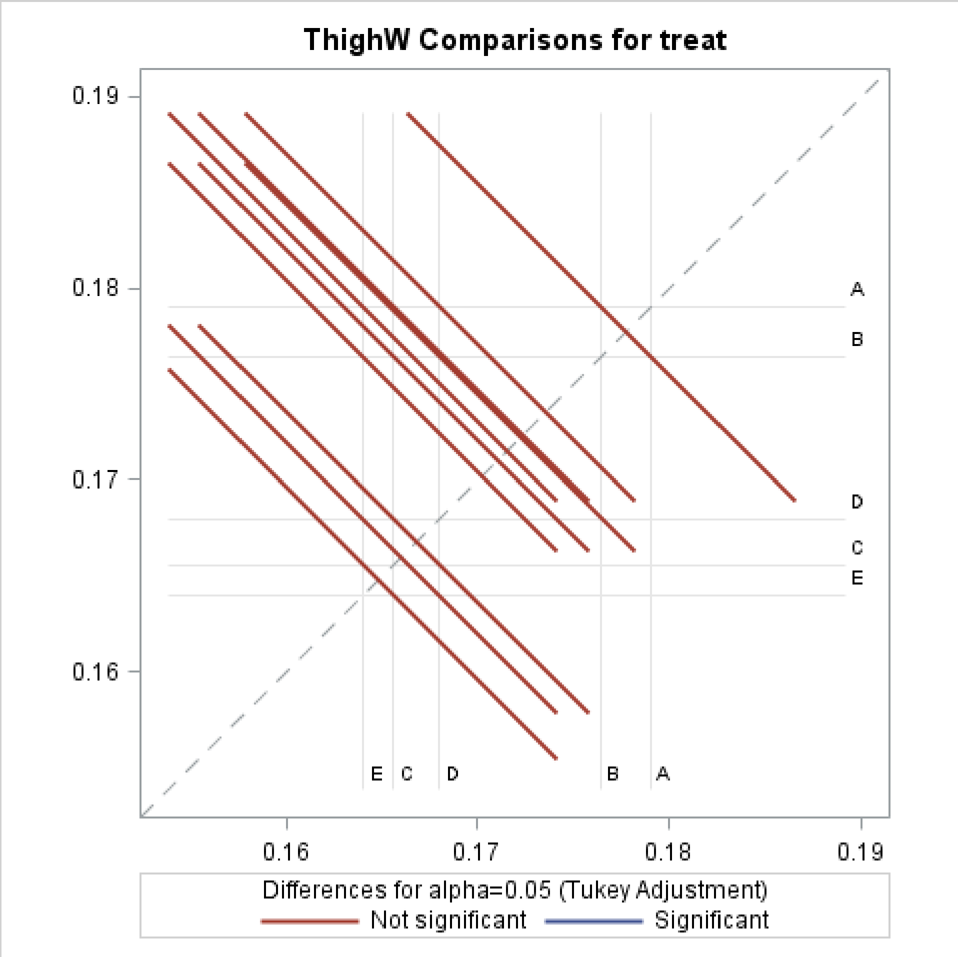
The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

treat	ThighW LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	0.17900000	0.00479416	<.0001	1
B	0.17640000	0.00479416	<.0001	2
C	0.16560000	0.00479416	<.0001	3
D	0.16800000	0.00479416	<.0001	4
E	0.16400000	0.00479416	<.0001	5

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: ThighW

i/j	1	2	3	4	5
1		0.9950	0.3124	0.5010	0.2155
2	0.9950		0.5184	0.7295	0.3855
3	0.3124	0.5184		0.9963	0.9993
4	0.5010	0.7295	0.9963		0.9750
5	0.2155	0.3855	0.9993	0.9750	


LS-Means for treat
0.180
0.175
0.170
0.165
ThighW LS-Mean
A
B
C
D
E
treat



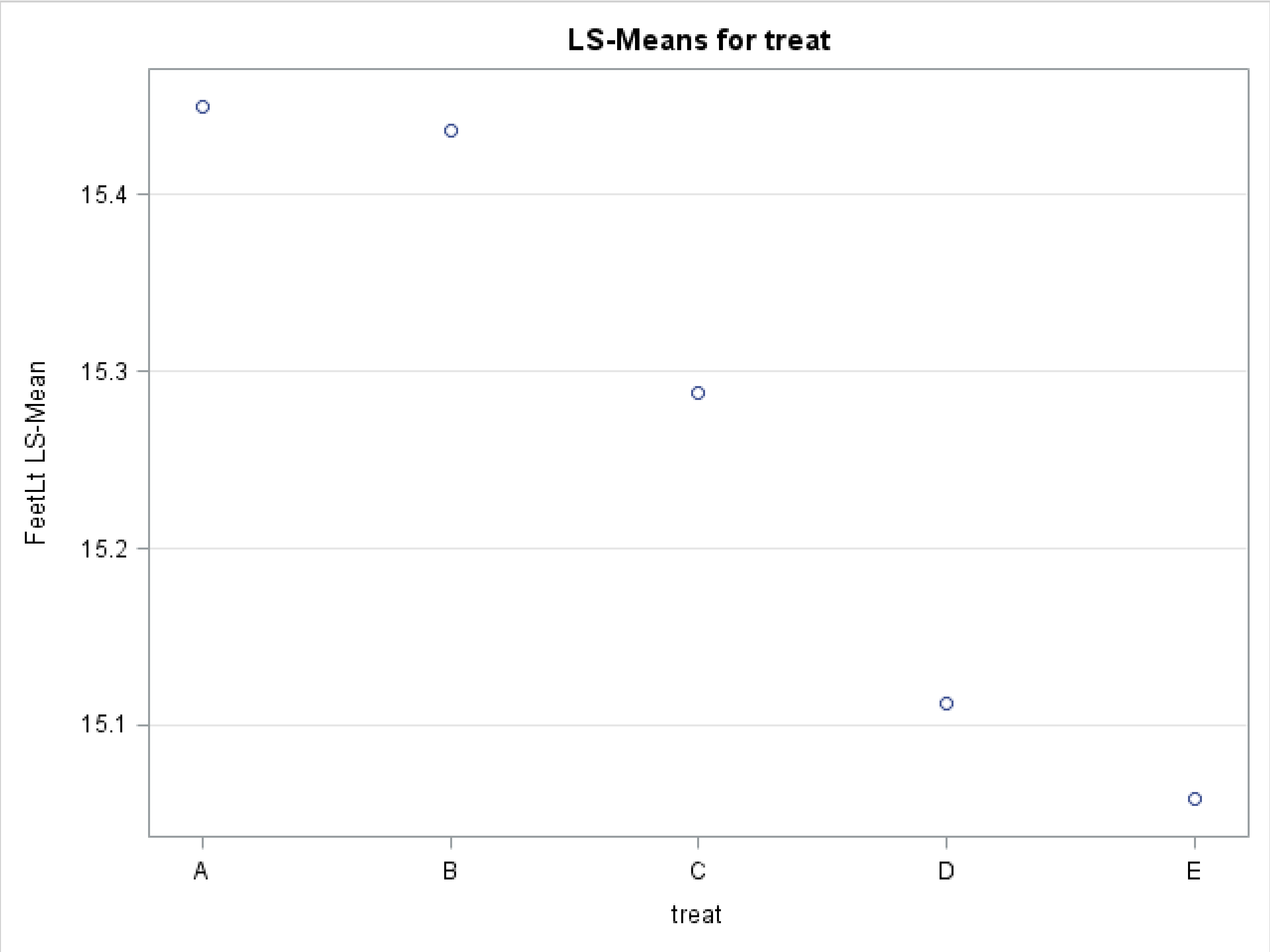
treat	FeetLt LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	15.4500000	0.4018527	<.0001	1
B	15.4360000	0.4018527	<.0001	2
C	15.2880000	0.4018527	<.0001	3
D	15.1120000	0.4018527	<.0001	4
E	15.0580000	0.4018527	<.0001	5

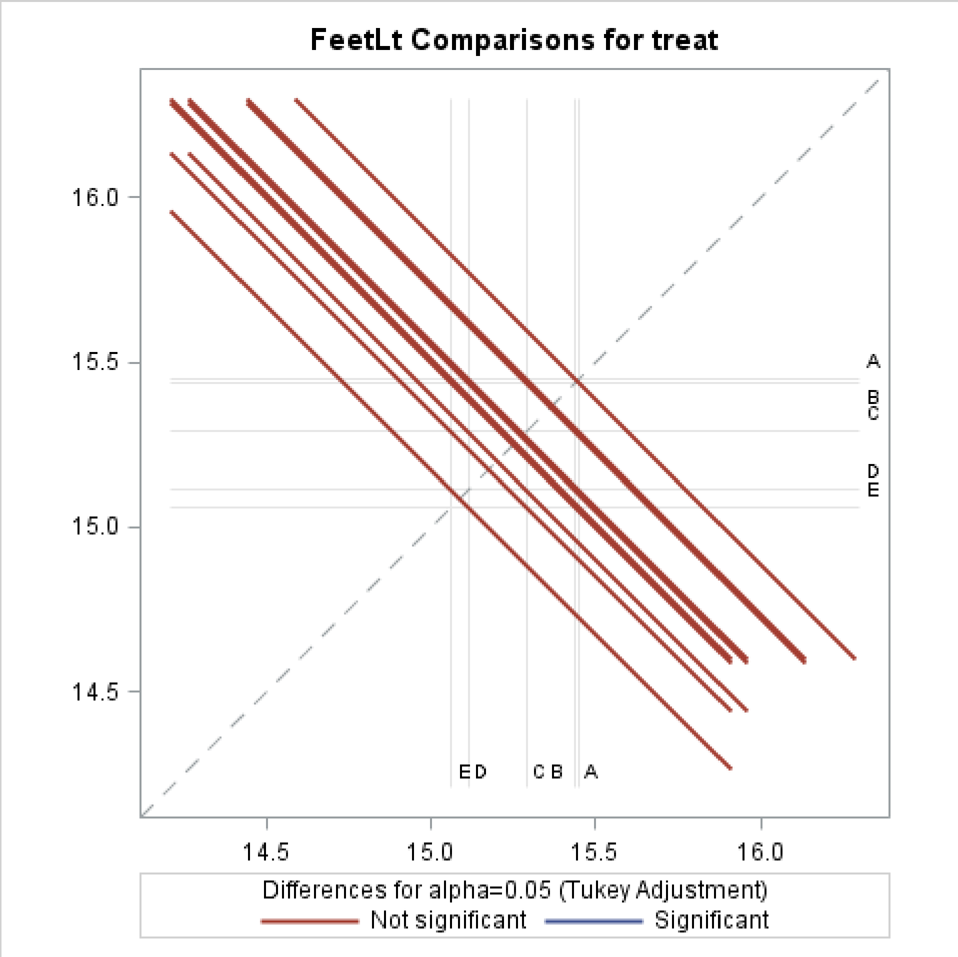
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: FeetLt

i/j	1	2	3	4	5
1		1.0000	0.9984	0.9743	0.9564
2	1.0000		0.9989	0.9780	0.9616
3	0.9984	0.9989		0.9978	0.9939
4	0.9743	0.9780	0.9978		1.0000

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: FeetLt

i/j	1	2	3	4	5
5	0.9564	0.9616	0.9939	1.0000	





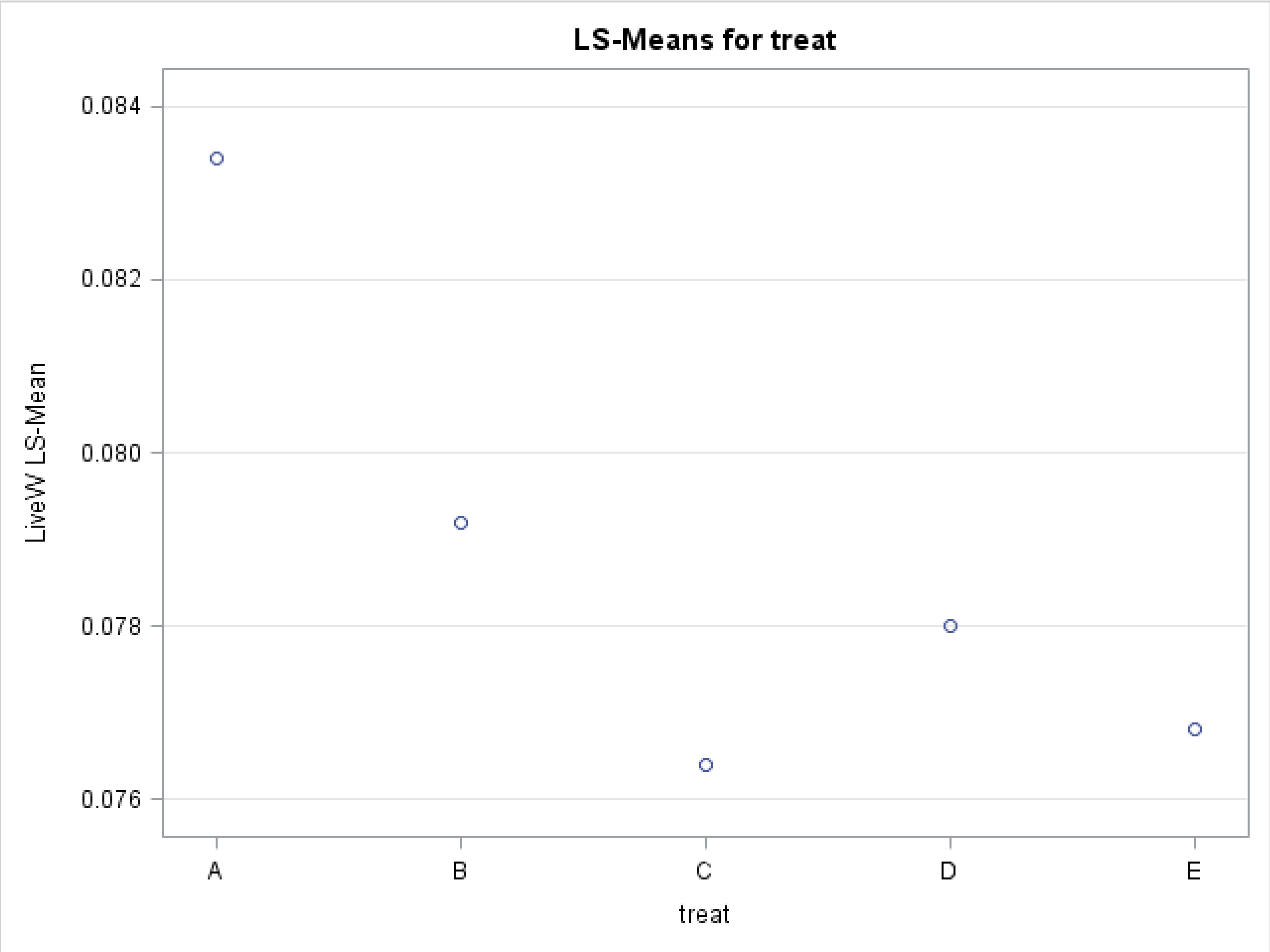
treat	LiveW LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	0.08340000	0.00384968	<.0001	1
B	0.07920000	0.00384968	<.0001	2
C	0.07640000	0.00384968	<.0001	3
D	0.07800000	0.00384968	<.0001	4
E	0.07680000	0.00384968	<.0001	5

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: LiveW

i/j	1	2	3	4	5
1		0.9359	0.7025	0.8559	0.7445
2	0.9359		0.9849	0.9994	0.9915
3	0.7025	0.9849		0.9982	1.0000
4	0.8559	0.9994	0.9982		0.9994

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: LiveW

i/j	1	2	3	4	5
5	0.7445	0.9915	1.0000	0.9994	



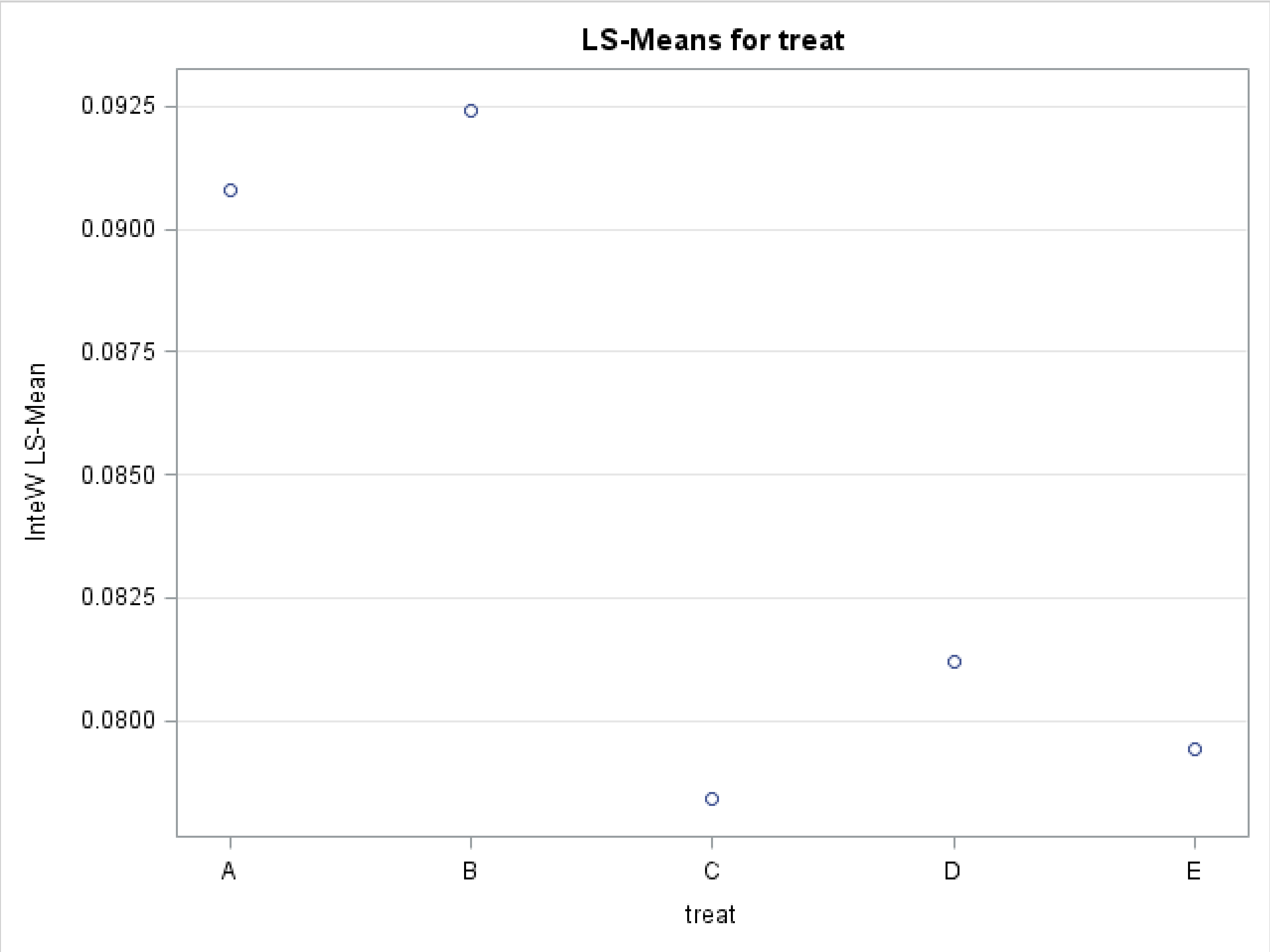
treat	InteW LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	0.09080000	0.00437173	<.0001	1
B	0.09240000	0.00437173	<.0001	2
C	0.07840000	0.00437173	<.0001	3
D	0.08120000	0.00437173	<.0001	4
E	0.07940000	0.00437173	<.0001	5

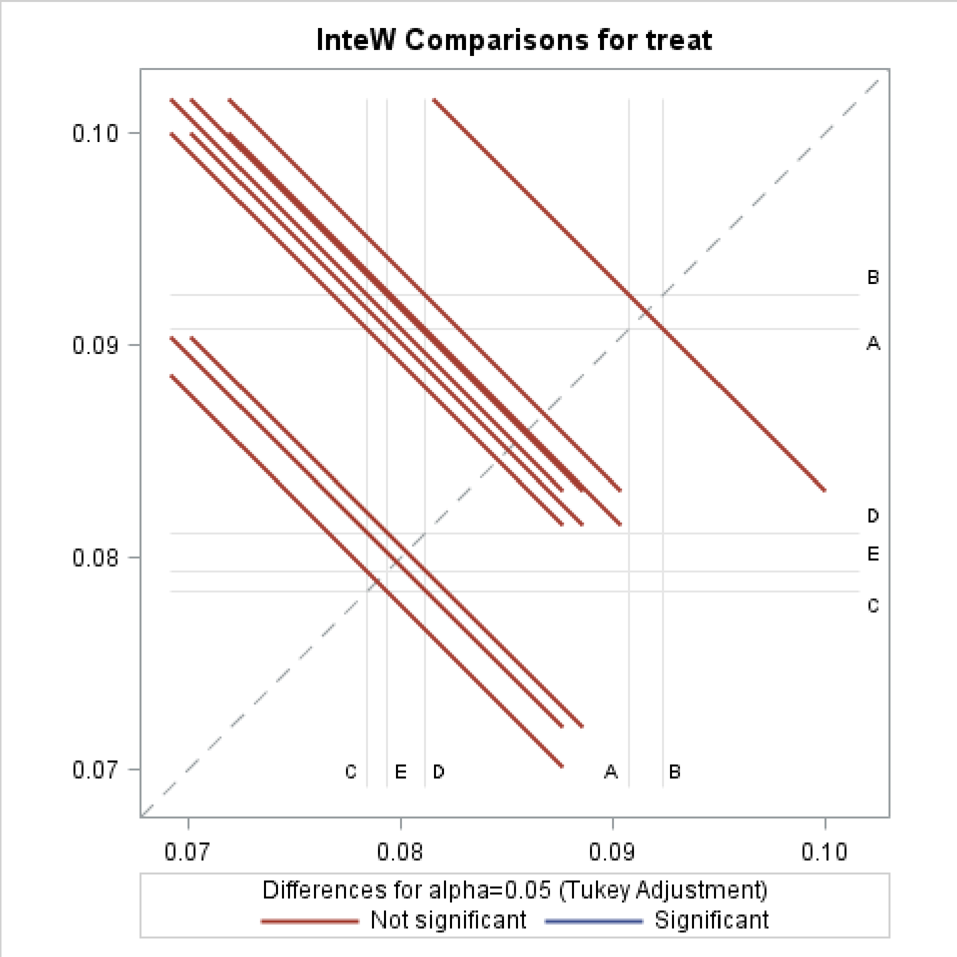
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: InteW

i/j	1	2	3	4	5
1		0.9989	0.2991	0.5424	0.3777
2	0.9989		0.1975	0.3947	0.2574
3	0.2991	0.1975		0.9906	0.9998
4	0.5424	0.3947	0.9906		0.9983

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: InteW

i/j	1	2	3	4	5
5	0.3777	0.2574	0.9998	0.9983	





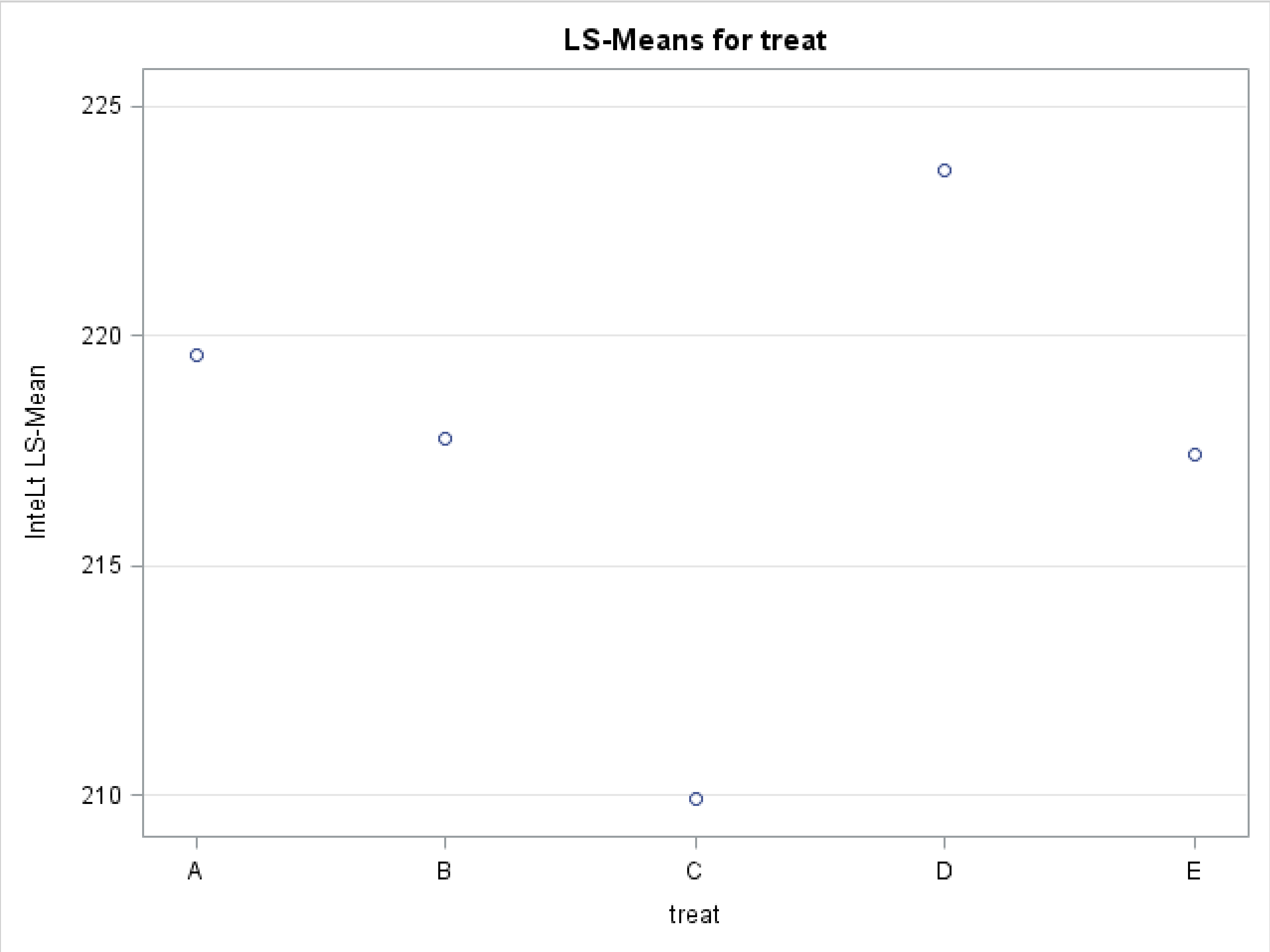
treat	InteLt LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	219.588000	6.194231	<.0001	1
B	217.756000	6.194231	<.0001	2
C	209.924000	6.194231	<.0001	3
D	223.630000	6.194231	<.0001	4
E	217.418000	6.194231	<.0001	5

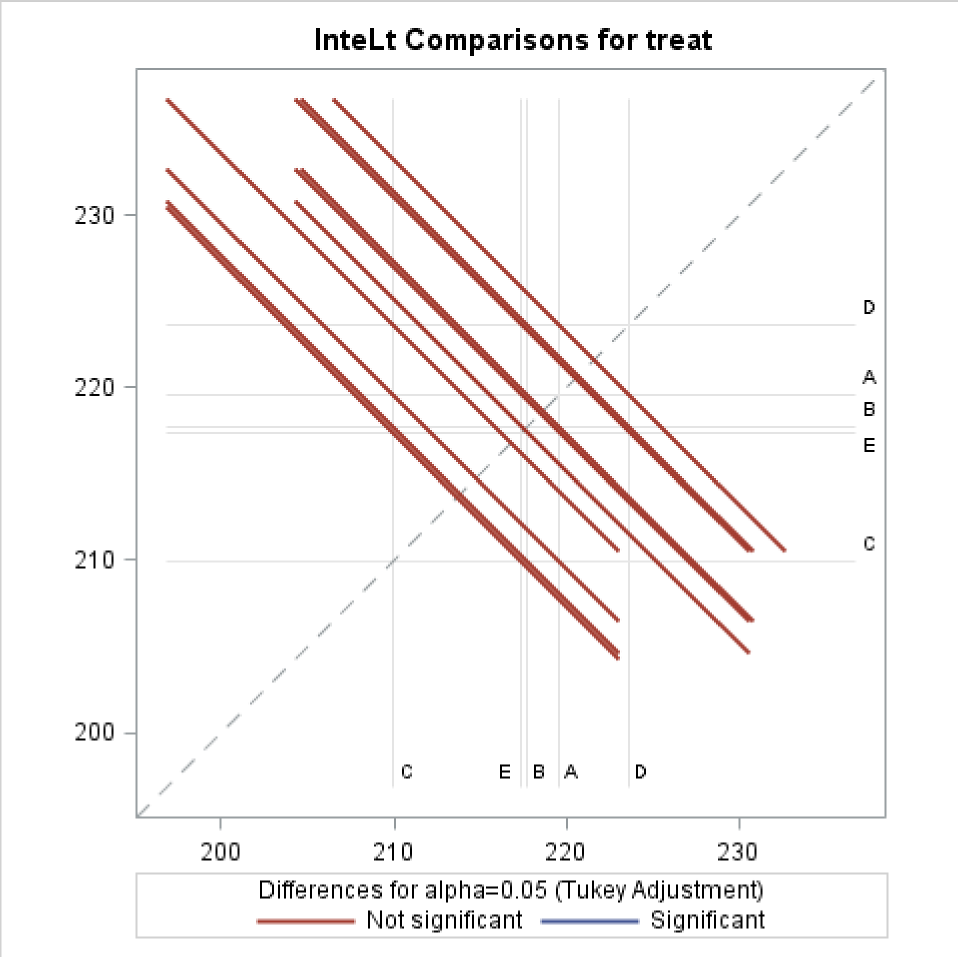
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: InteLt

i/j	1	2	3	4	5
1		0.9995	0.8029	0.9900	0.9991
2	0.9995		0.8957	0.9605	1.0000
3	0.8029	0.8957		0.5353	0.9096
4	0.9900	0.9605	0.5353		0.9520

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: InteLt

i/j	1	2	3	4	5
5	0.9991	1.0000	0.9096	0.9520	





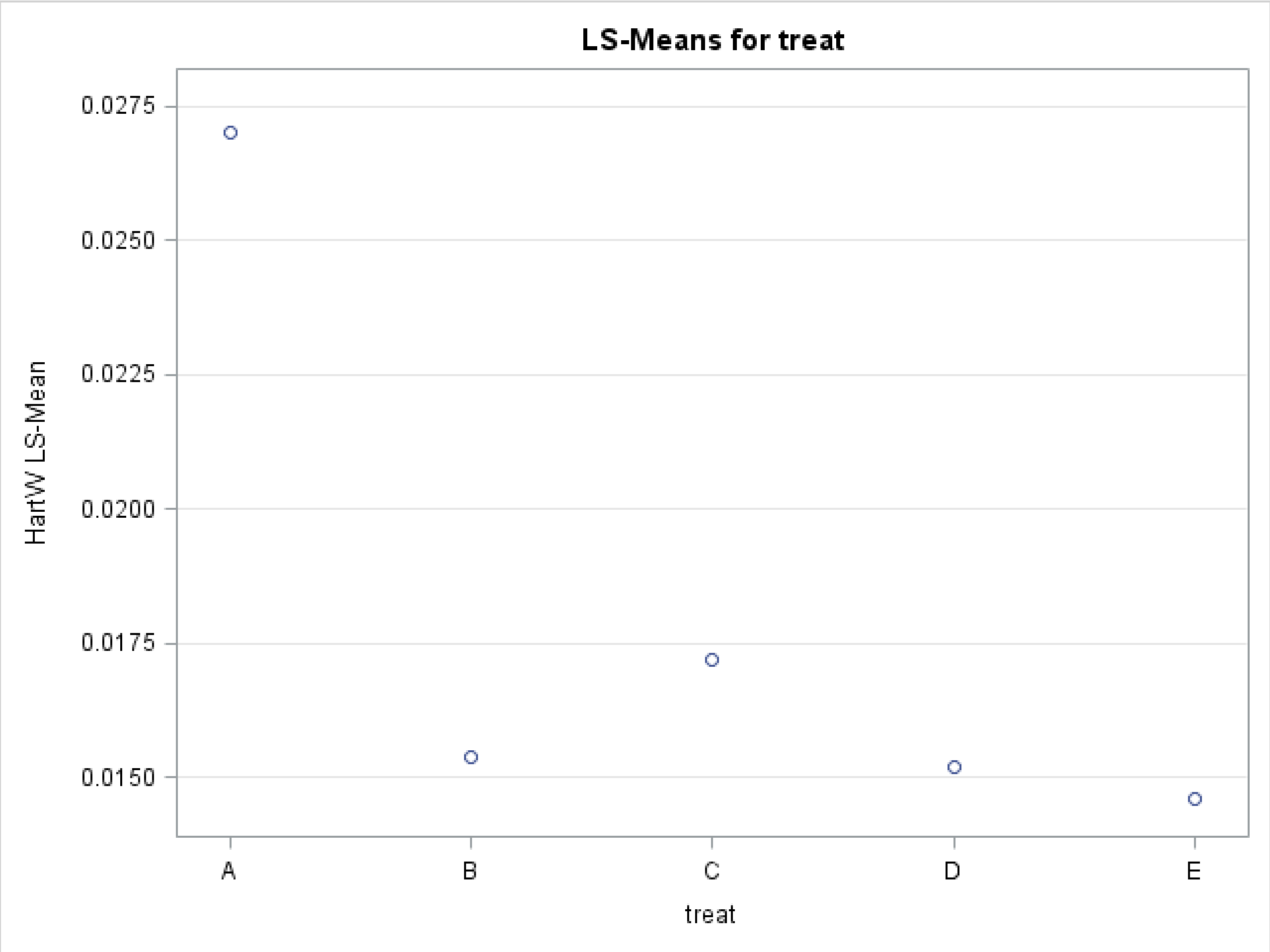
treat	HartW LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	0.02700000	0.00384708	<.0001	1
B	0.01540000	0.00384708	0.0007	2
C	0.01720000	0.00384708	0.0002	3
D	0.01520000	0.00384708	0.0008	4
E	0.01460000	0.00384708	0.0011	5

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: HartW

i/j	1	2	3	4	5
1		0.2457	0.4001	0.2315	0.1927
2	0.2457		0.9972	1.0000	0.9999
3	0.4001	0.9972		0.9958	0.9885
4	0.2315	1.0000	0.9958		1.0000

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: HartW

i/j	1	2	3	4	5
5	0.1927	0.9999	0.9885	1.0000	



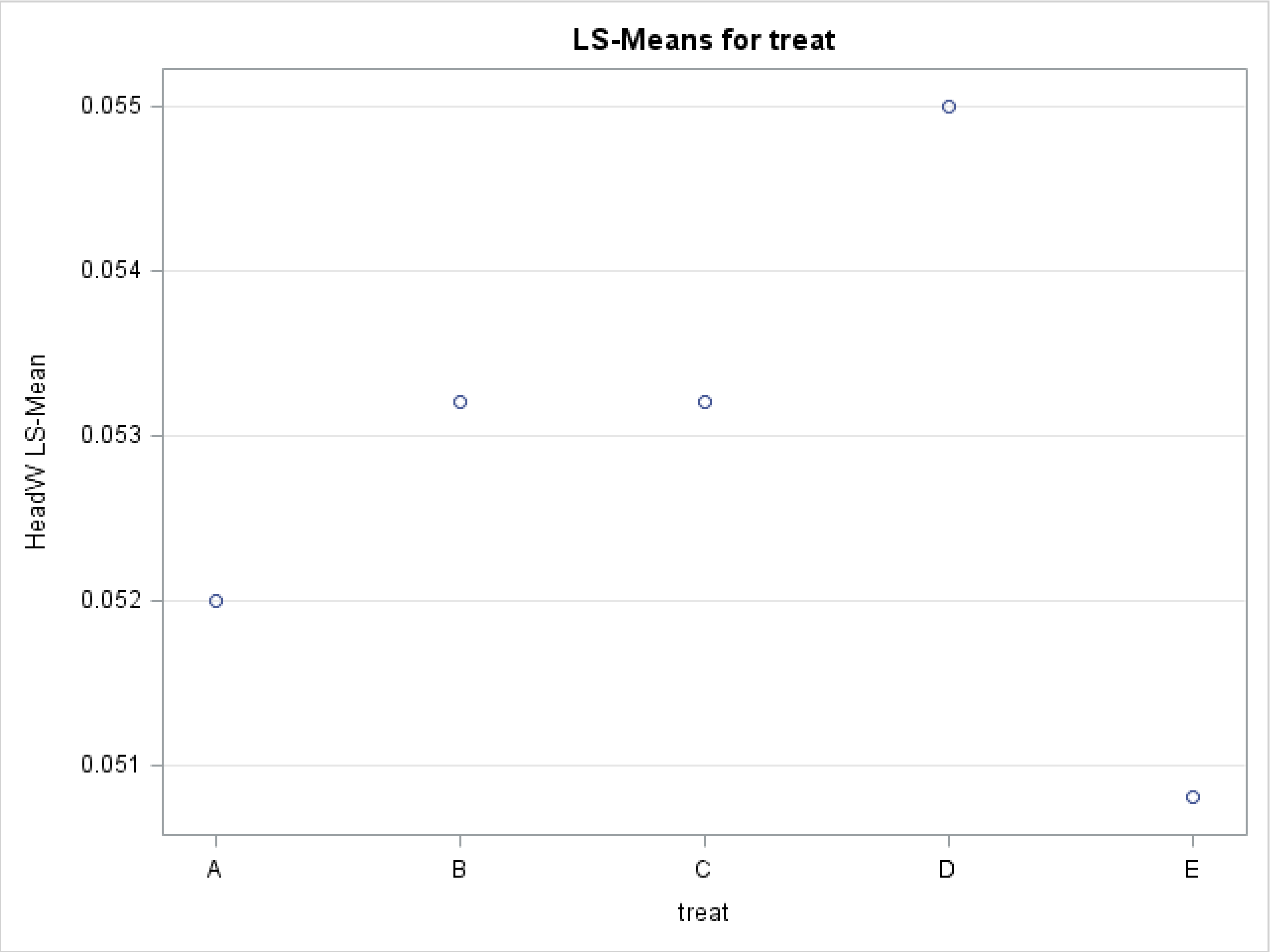
treat	HeadW LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	0.05200000	0.00228123	<.0001	1
B	0.05320000	0.00228123	<.0001	2
C	0.05320000	0.00228123	<.0001	3
D	0.05500000	0.00228123	<.0001	4
E	0.05080000	0.00228123	<.0001	5

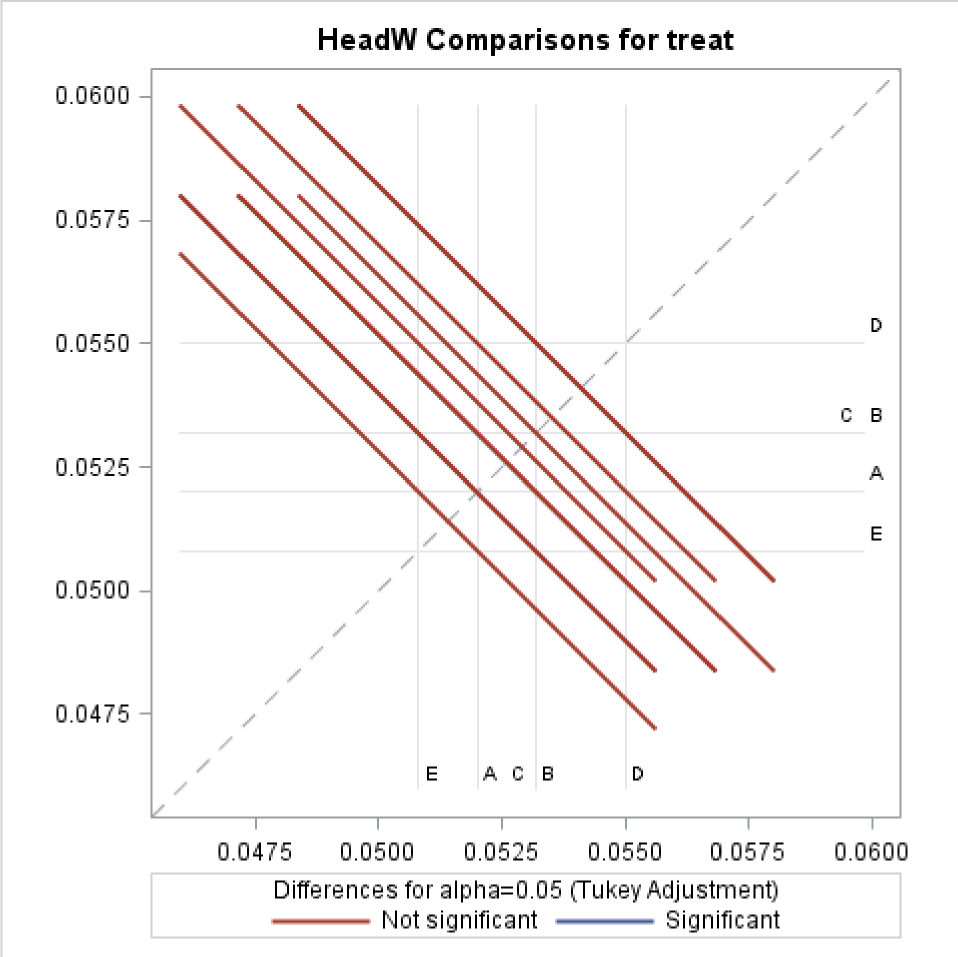
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: HeadW

i/j	1	2	3	4	5
1		0.9956	0.9956	0.8819	0.9956
2	0.9956		1.0000	0.9796	0.9434
3	0.9956	1.0000		0.9796	0.9434
4	0.8819	0.9796	0.9796		0.6931

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: HeadW

i/j	1	2	3	4	5
5	0.9956	0.9434	0.9434	0.6931	




HeadW Comparisons for treat
0.0600
0.0575
0.0550
0.0525
0.0500
0.0475
D
C B
A
E
E A C B D
0.0475 0.0500 0.0525 0.0550 0.0575 0.0600
Differences for alpha=0.05 (Tukey Adjustment)
Not significant
Significant

The GLM Procedure

Class Level Information

Class	Levels	Values
treat	5	A B C D E

Number of Observations Read	25
Number of Observations Used	25

The SAS System

The GLM Procedure

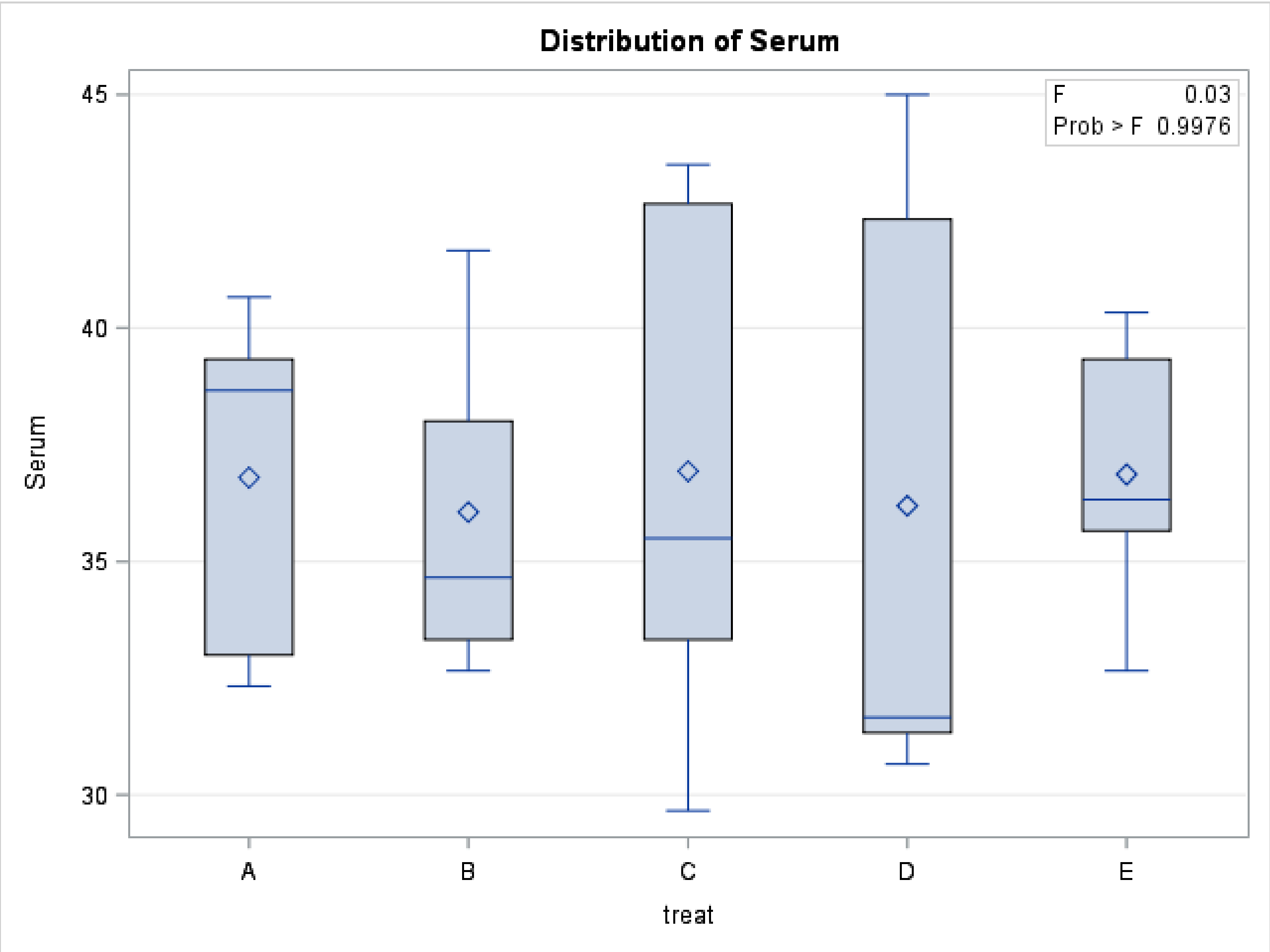
Dependent Variable: Serum

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	3.3193840	0.8298460	0.03	0.9976
Error	20	486.6726000	24.3336300		
Corrected Total	24	489.9919840			

R-Square	Coeff Var	Root MSE	Serum Mean
0.006774	13.48926	4.932913	36.56920

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	3.31938400	0.82984600	0.03	0.9976

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	3.31938400	0.82984600	0.03	0.9976



The GLM Procedure

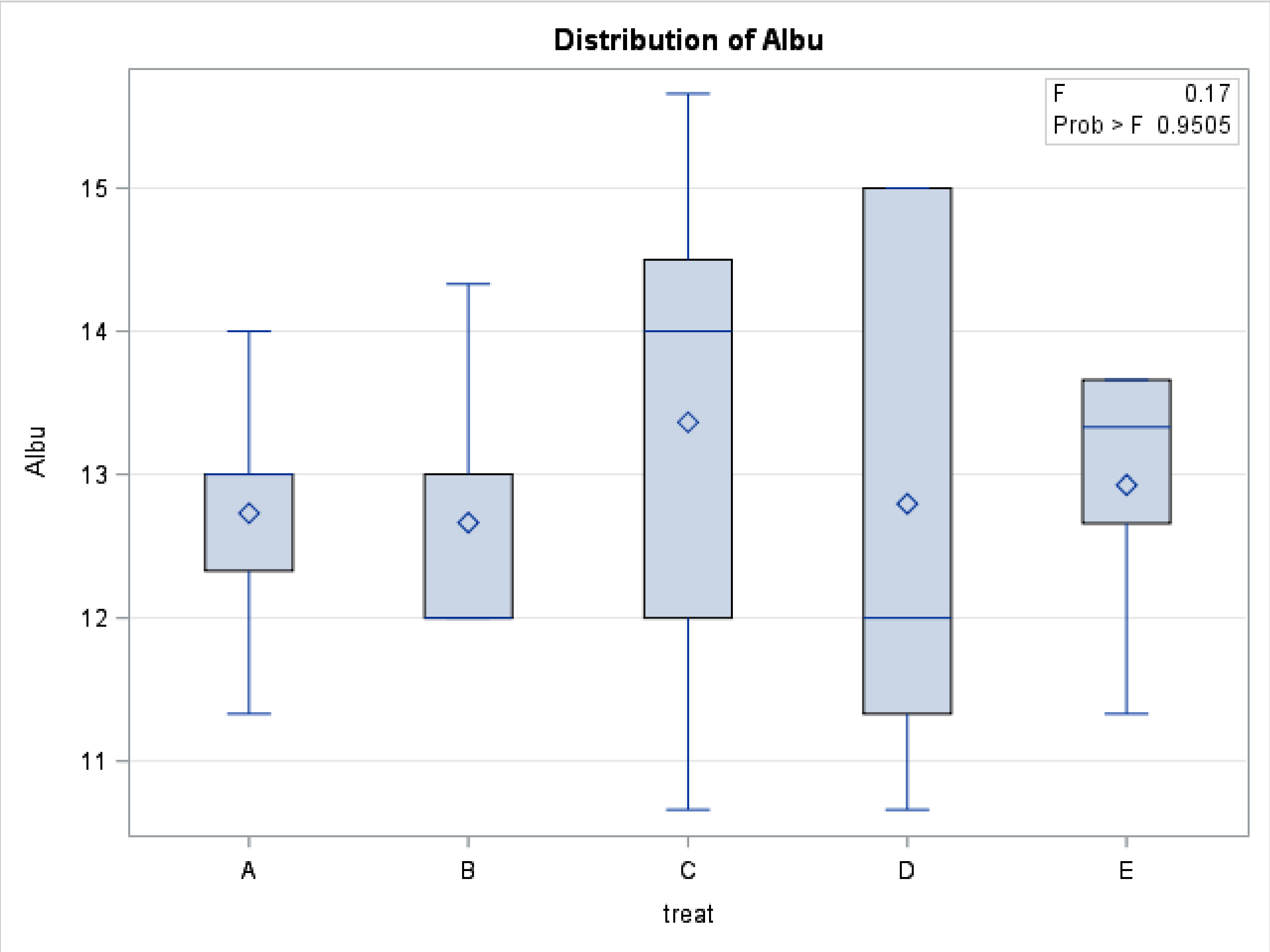
Dependent Variable: Albu

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1.54717600	0.38679400	0.17	0.9505
Error	20	45.14768000	2.25738400		
Corrected Total	24	46.69485600			

R-Square	Coeff Var	Root MSE	Albu Mean
0.033134	11.64914	1.502459	12.89760

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	1.54717600	0.38679400	0.17	0.9505

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	1.54717600	0.38679400	0.17	0.9505



The GLM Procedure

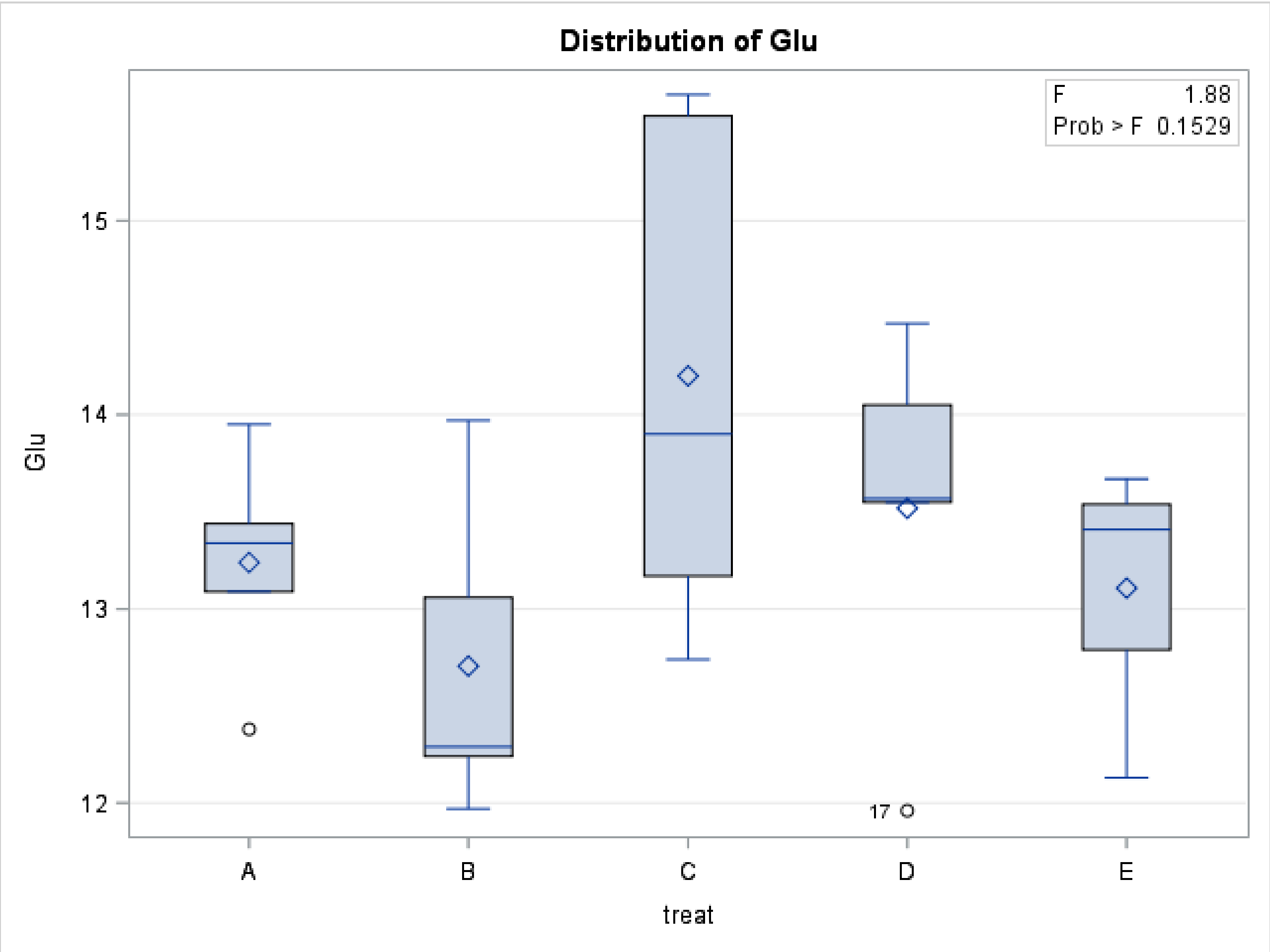
Dependent Variable: Glu

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	6.18342400	1.54585600	1.88	0.1529
Error	20	16.42340000	0.82117000		
Corrected Total	24	22.60682400			

R-Square	Coeff Var	Root MSE	Glu Mean
0.273520	6.785458	0.906184	13.35480

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	6.18342400	1.54585600	1.88	0.1529

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	6.18342400	1.54585600	1.88	0.1529



The SAS System

The GLM Procedure

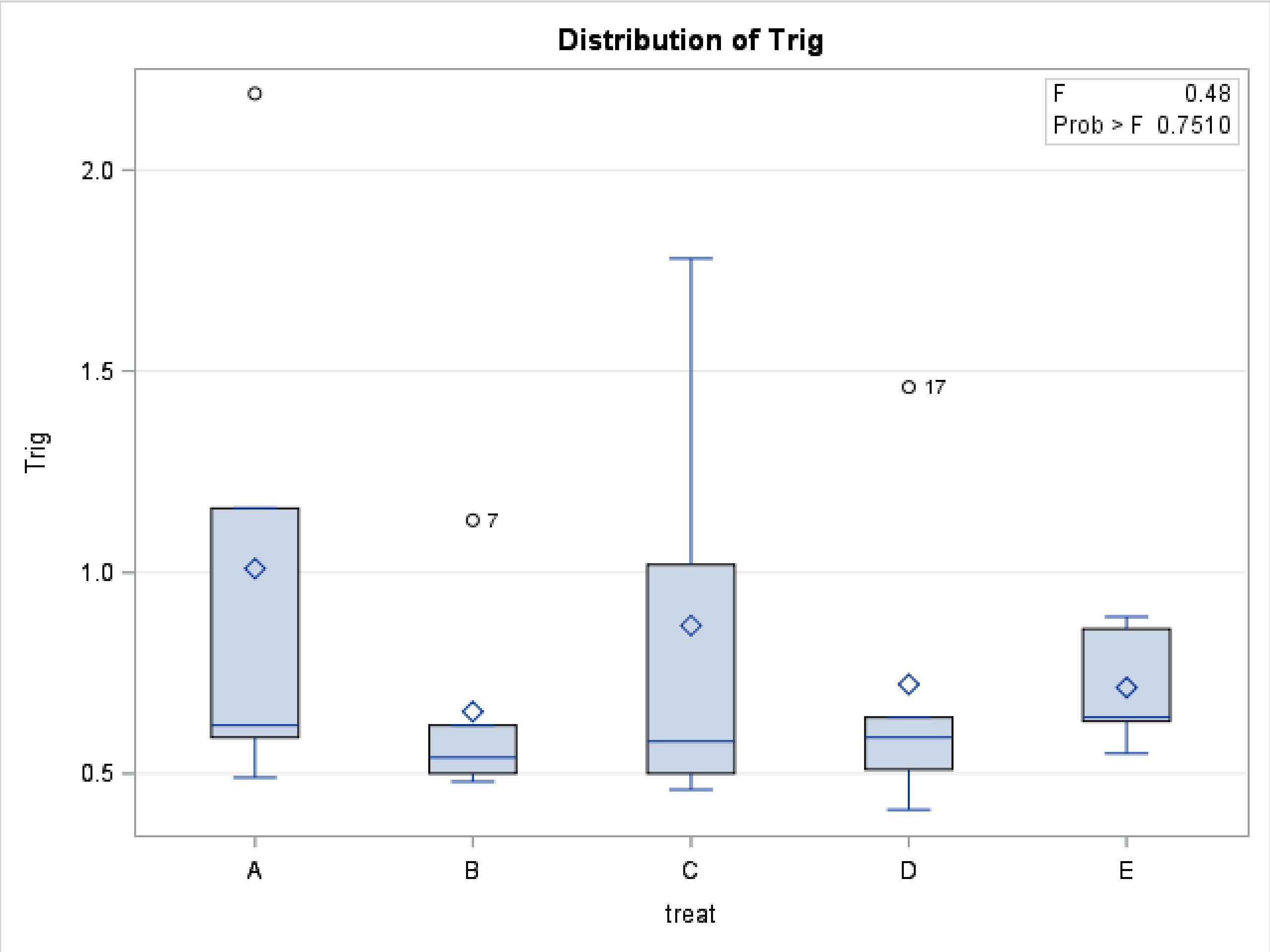
Dependent Variable: Trig

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.41657600	0.10414400	0.48	0.7510
Error	20	4.35100000	0.21755000		
Corrected Total	24	4.76757600			

R-Square	Coeff Var	Root MSE	Trig Mean
0.087377	58.77300	0.466423	0.793600

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	0.41657600	0.10414400	0.48	0.7510

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	0.41657600	0.10414400	0.48	0.7510



The GLM Procedure

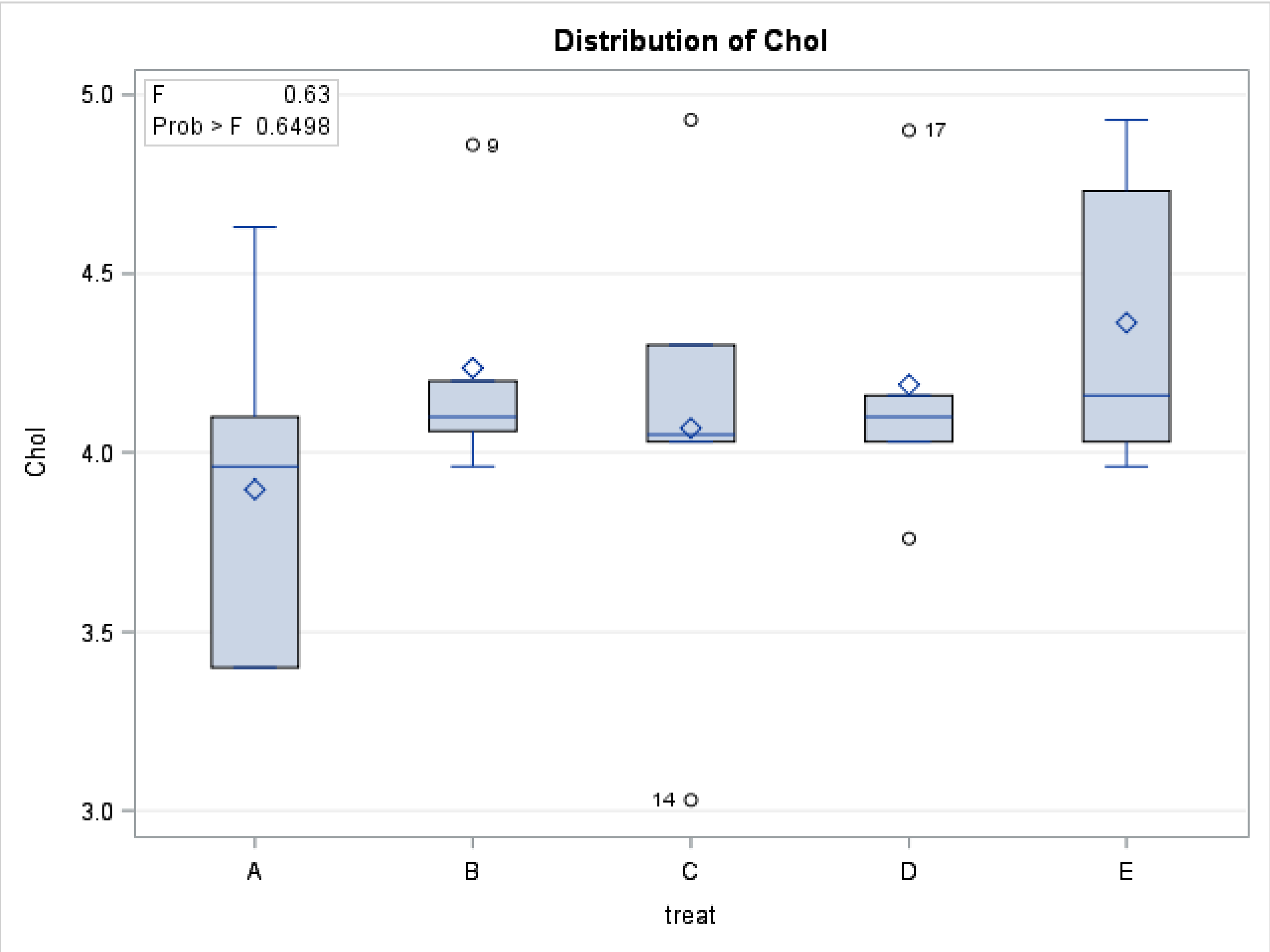
Dependent Variable: Chol

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.62082400	0.15520600	0.63	0.6498
Error	20	4.96316000	0.24815800		
Corrected Total	24	5.58398400			

R-Square	Coeff Var	Root MSE	Chol Mean
0.111179	12.00141	0.498155	4.150800

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	0.62082400	0.15520600	0.63	0.6498

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	0.62082400	0.15520600	0.63	0.6498



The GLM Procedure

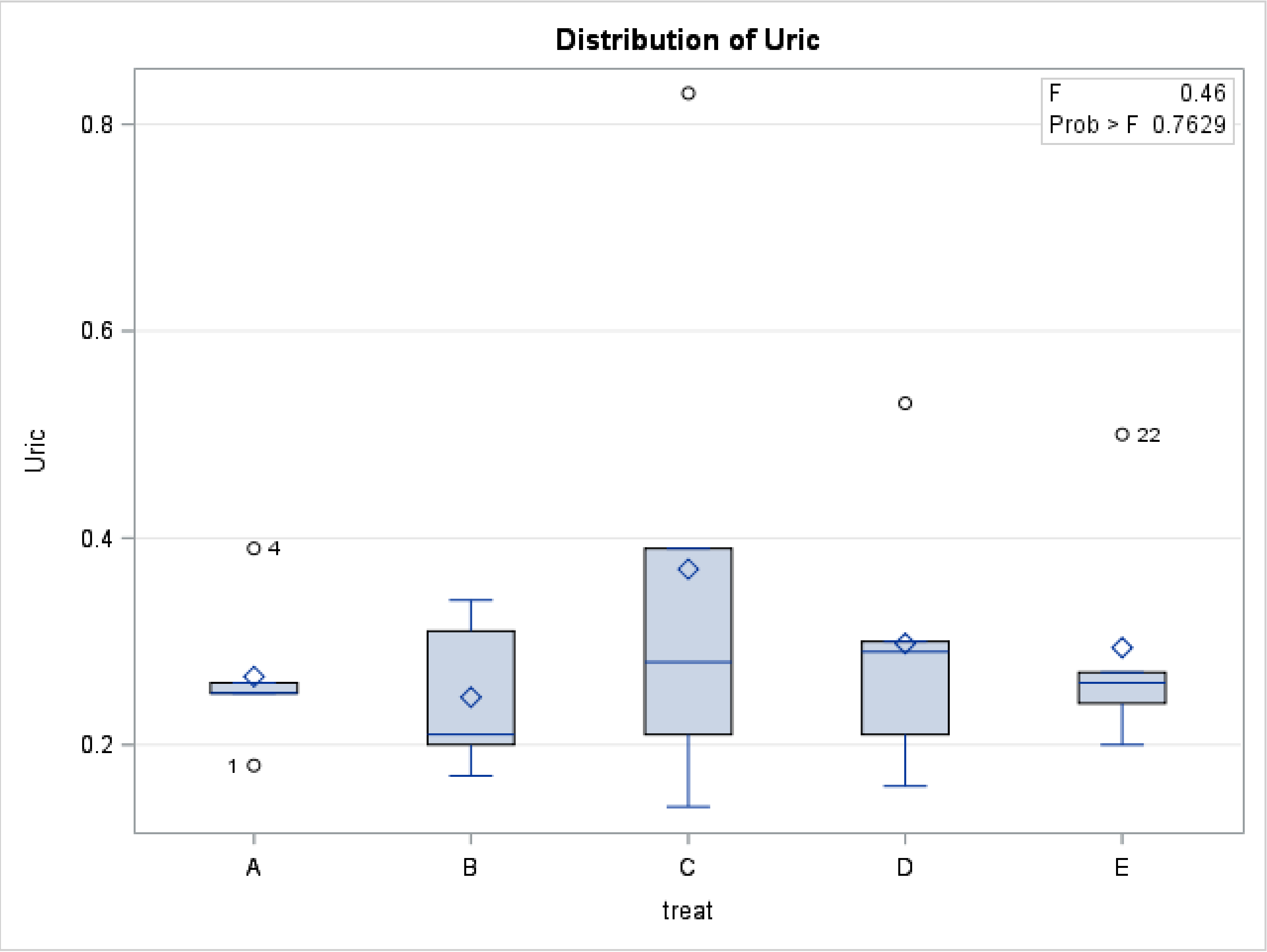
Dependent Variable: Uric

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.04438400	0.01109600	0.46	0.7629
Error	20	0.48064000	0.02403200		
Corrected Total	24	0.52502400			

R-Square	Coeff Var	Root MSE	Uric Mean
0.084537	52.58568	0.155023	0.294800

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	0.04438400	0.01109600	0.46	0.7629

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	0.04438400	0.01109600	0.46	0.7629



The SAS System

The GLM Procedure

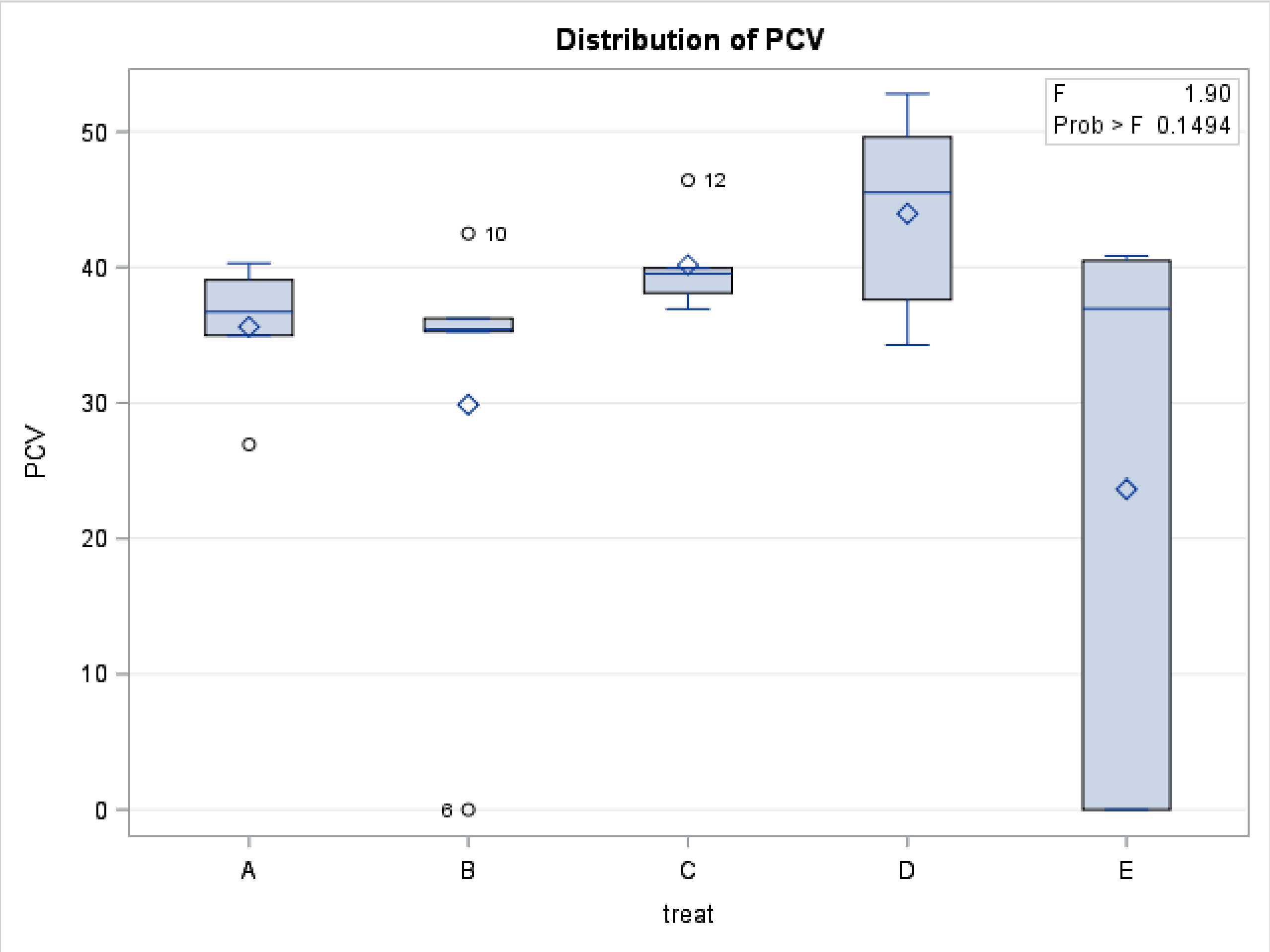
Dependent Variable: PCV

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	1308.287064	327.071766	1.90	0.1494
Error	20	3438.222880	171.911144		
Corrected Total	24	4746.509944			

R-Square	Coeff Var	Root MSE	PCV Mean
0.275631	37.84329	13.11149	34.64680

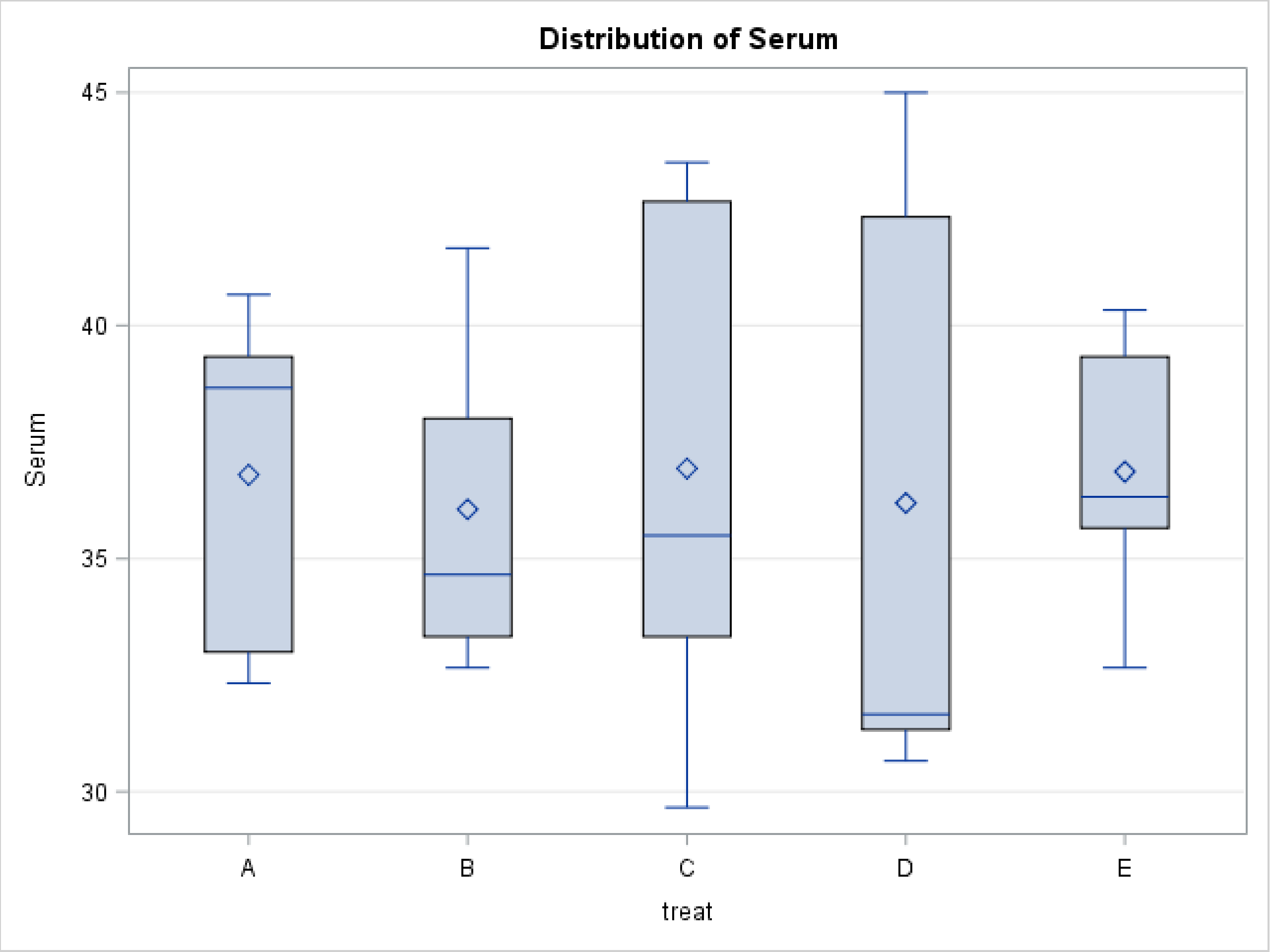
Source	DF	Type I SS	Mean Square	F Value	Pr > F
treat	4	1308.287064	327.071766	1.90	0.1494

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treat	4	1308.287064	327.071766	1.90	0.1494



The SAS System

The GLM Procedure



The GLM Procedure

t Tests (LSD) for Serum

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	24.33363
Critical Value of t	2.08596
Least Significant Difference	6.5079

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	36.930	5	C
A			
A	36.862	5	E
A			
A	36.796	5	A
A			
A	36.196	5	D
A			
A	36.062	5	B

The SAS System

The GLM Procedure

Duncan's Multiple Range Test for Serum

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	24.33363

Number of Means	**2**	**3**	**4**	**5**
Critical Range	6.508	6.831	7.037	7.180

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treat
A	36.930	5	C
A			
A	36.862	5	E
A			
A	36.796	5	A
A			
A	36.196	5	D
A			
A	36.062	5	B

The GLM Procedure

Tukey's Studentized Range (HSD) Test for Serum

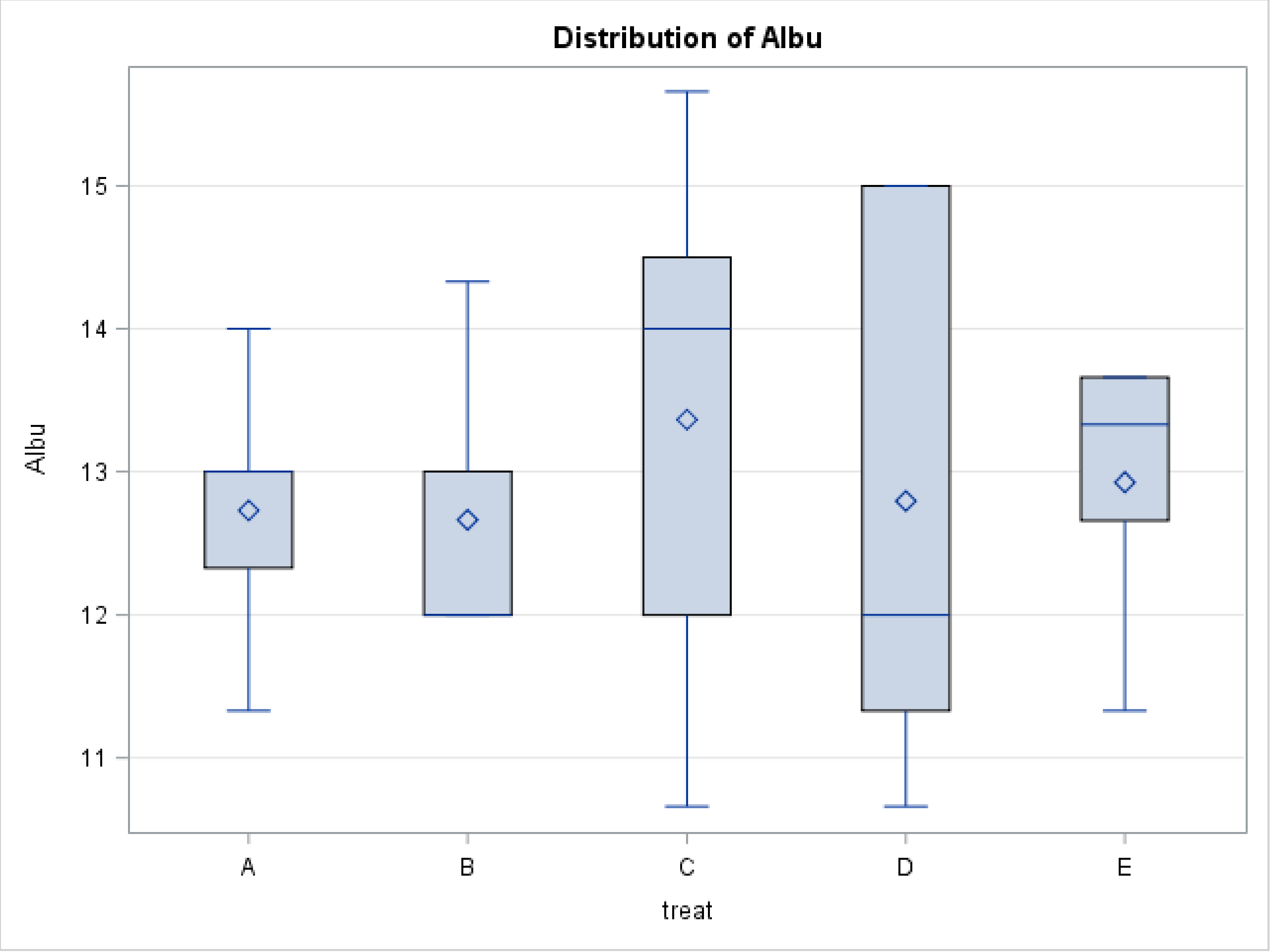
Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	24.33363
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	9.3358

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	36.930	5	C
A			
A	36.862	5	E
A			
A	36.796	5	A
A			
A	36.196	5	D
A			
A	36.062	5	B

The GLM Procedure



The GLM Procedure

t Tests (LSD) for Albu

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	2.257384
Critical Value of t	2.08596
Least Significant Difference	1.9822

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	13.3640	5	C
A			
A	12.9280	5	E
A			
A	12.7980	5	D
A			
A	12.7320	5	A
A			
A	12.6660	5	B

The SAS System

The GLM Procedure

Duncan's Multiple Range Test for Albu

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	2.257384

Number of Means	**2**	**3**	**4**	**5**
Critical Range	1.982	2.081	2.143	2.187

Means with the same letter are not significantly different.

Duncan Grouping	**Mean**	**N**	**treat**
A	13.3640	5	C
A			
A	12.9280	5	E
A			
A	12.7980	5	D
A			
A	12.7320	5	A
A			
A	12.6660	5	B

The GLM Procedure

Tukey's Studentized Range (HSD) Test for Albu

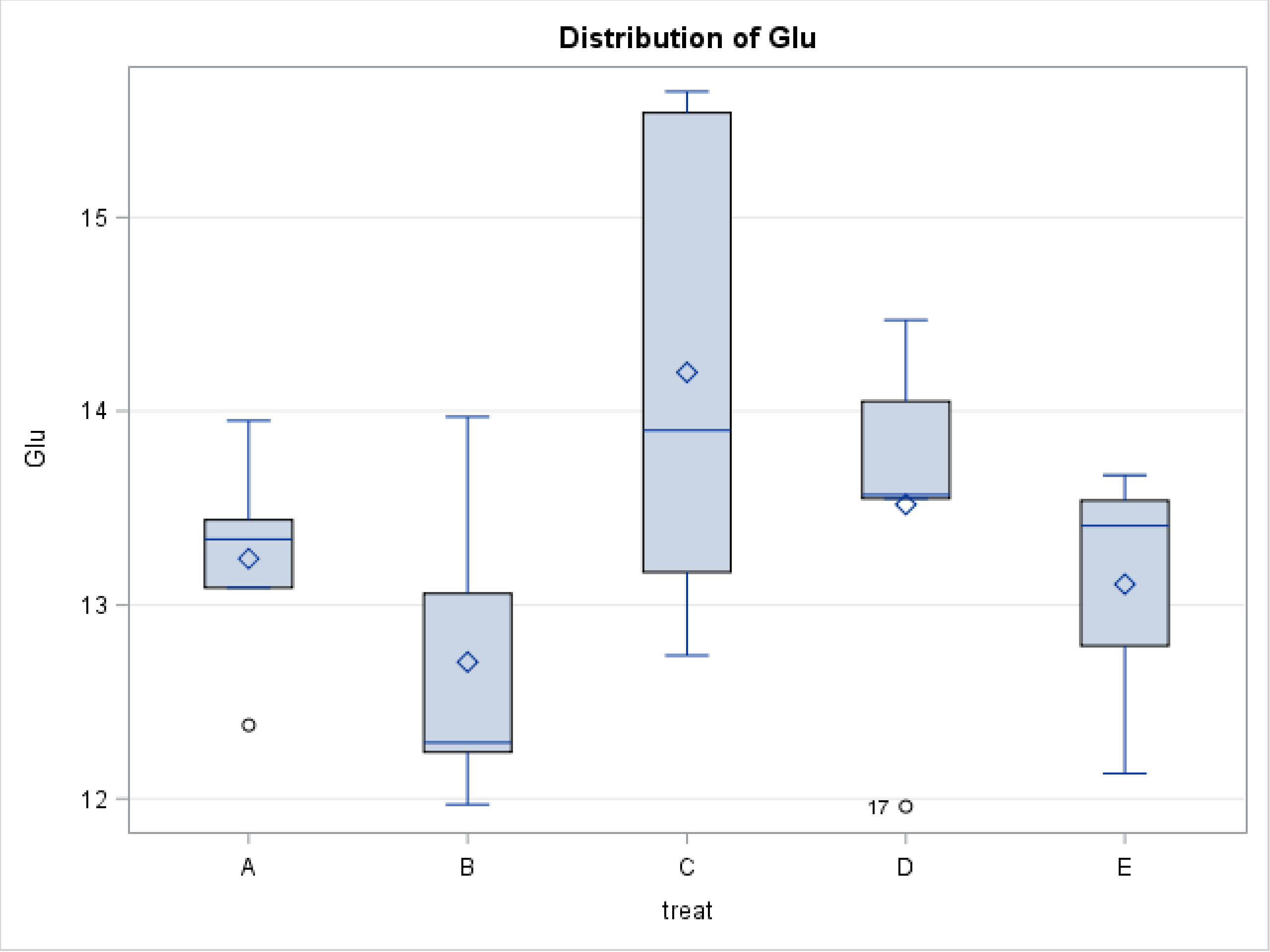
Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	2.257384
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	2.8435

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	13.3640	5	C
A			
A	12.9280	5	E
A			
A	12.7980	5	D
A			
A	12.7320	5	A
A			
A	12.6660	5	B

The GLM Procedure



The GLM Procedure

t Tests (LSD) for Glu

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.82117
Critical Value of t	2.08596
Least Significant Difference	1.1955

Means with the same letter are not significantly different.

t Grouping		Mean	N	treat
	A	14.2000	5	C
	A			
B	A	13.5200	5	D
B	A			
B	A	13.2400	5	A
B	A			
B	A	13.1080	5	E
B				
B		12.7060	5	B

The GLM Procedure

Duncan's Multiple Range Test for Glu

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.82117

Number of Means	**2**	**3**	**4**	**5**
Critical Range	1.195	1.255	1.293	1.319

Means with the same letter are not significantly different.

Duncan Grouping		**Mean**	**N**	**treat**
	A	14.2000	5	C
	A			
B	A	13.5200	5	D
B	A			
B	A	13.2400	5	A
B	A			
B	A	13.1080	5	E
B				
B		12.7060	5	B

The SAS System

The GLM Procedure

Tukey's Studentized Range (HSD) Test for Glu

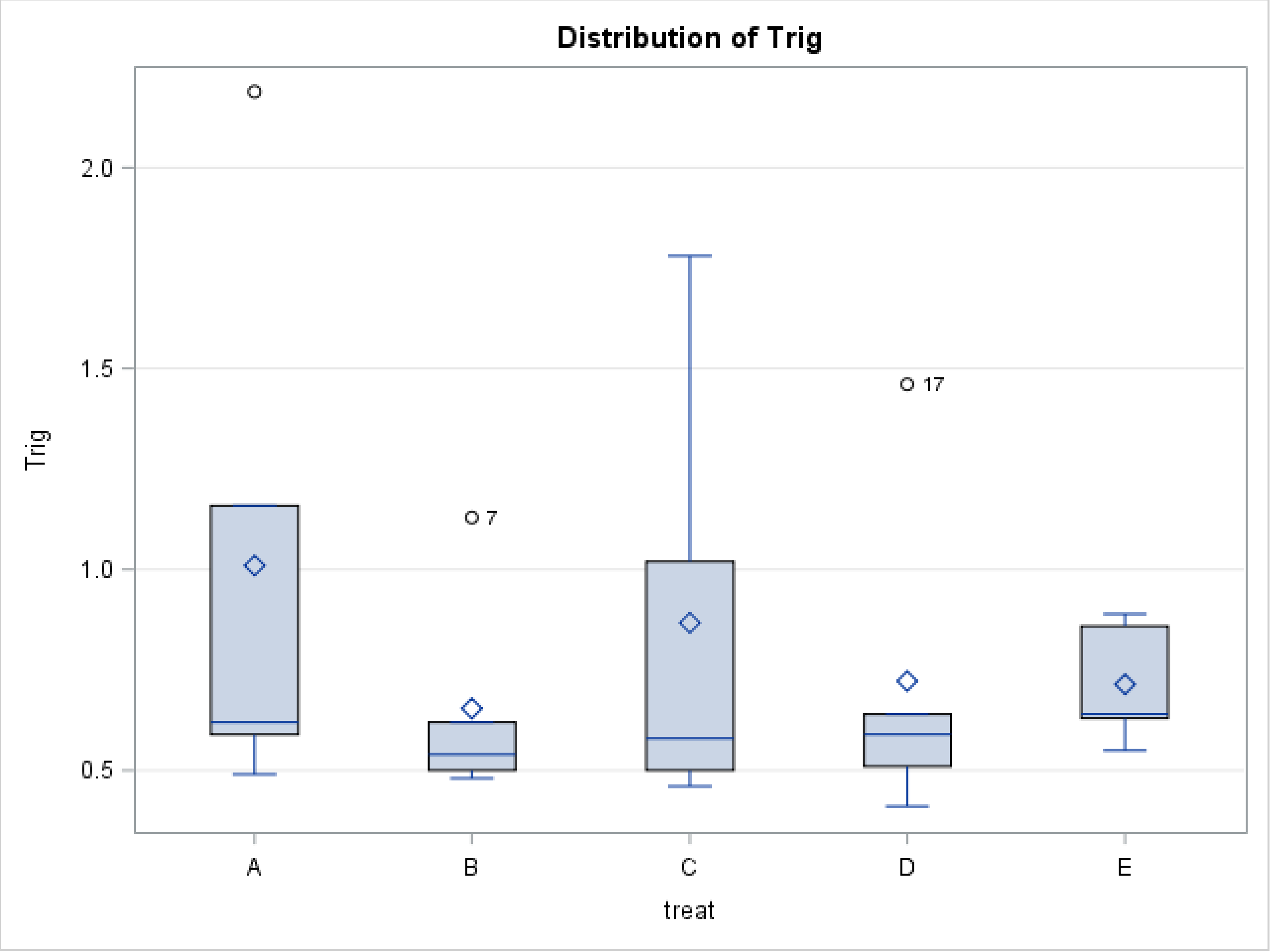
Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.82117
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	1.715

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	14.2000	5	C
A			
A	13.5200	5	D
A			
A	13.2400	5	A
A			
A	13.1080	5	E
A			
A	12.7060	5	B

The GLM Procedure



The GLM Procedure

t Tests (LSD) for Trig

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.21755
Critical Value of t	2.08596
Least Significant Difference	0.6153

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	1.0100	5	A
A			
A	0.8680	5	C
A			
A	0.7220	5	D
A			
A	0.7140	5	E
A			
A	0.6540	5	B

The GLM Procedure

Duncan's Multiple Range Test for Trig

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.21755

Number of Means	**2**	**3**	**4**	**5**
Critical Range	.6153	.6459	.6653	.6789

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	treat
A	1.0100	5	A
A			
A	0.8680	5	C
A			
A	0.7220	5	D
A			
A	0.7140	5	E
A			
A	0.6540	5	B

The GLM Procedure

Tukey's Studentized Range (HSD) Test for Trig

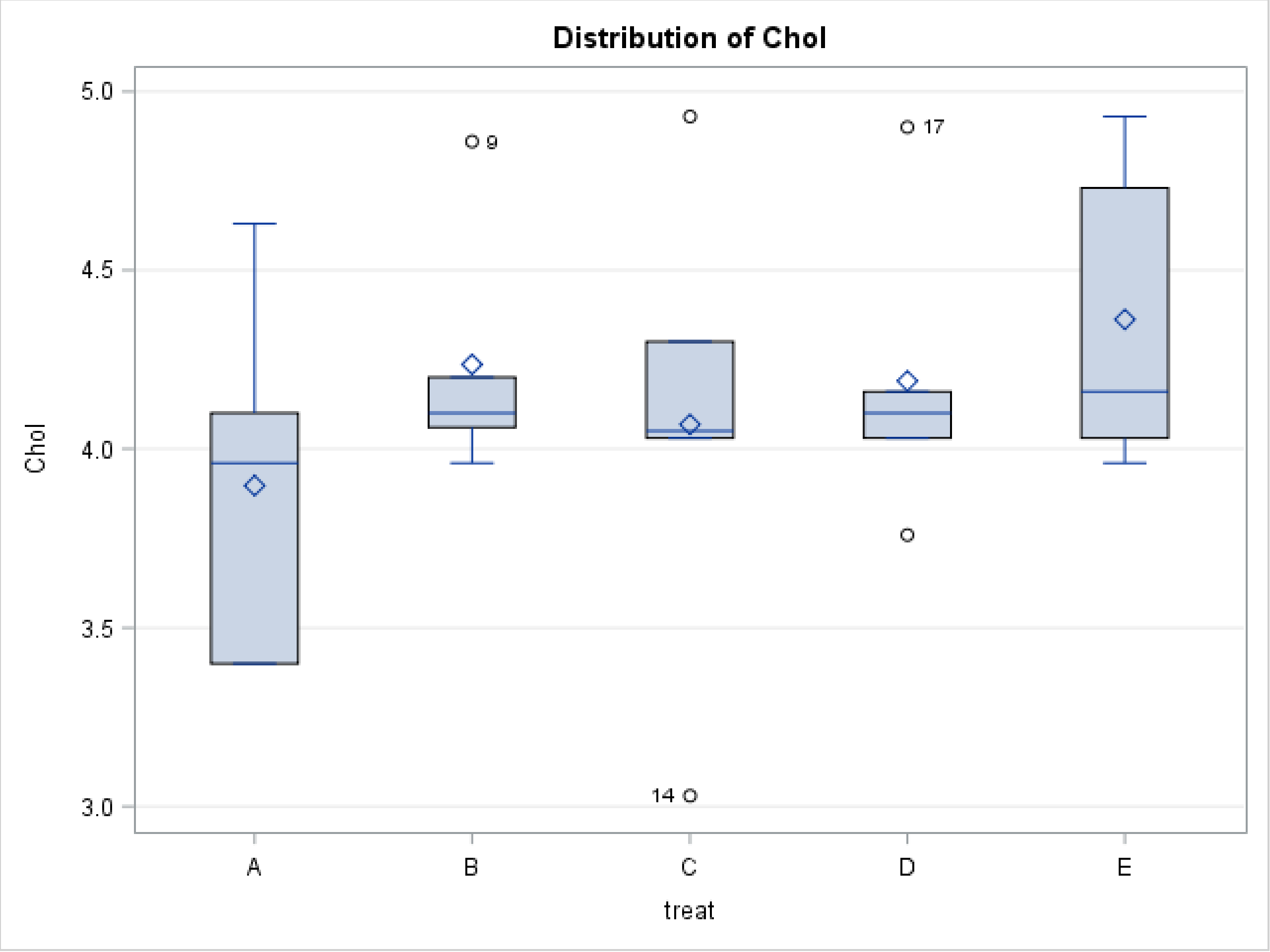
Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.21755
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.8827

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	1.0100	5	A
A			
A	0.8680	5	C
A			
A	0.7220	5	D
A			
A	0.7140	5	E
A			
A	0.6540	5	B

The GLM Procedure



The GLM Procedure

t Tests (LSD) for Chol

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.248158
Critical Value of t	2.08596
Least Significant Difference	0.6572

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	4.3620	5	E
A			
A	4.2360	5	B
A			
A	4.1900	5	D
A			
A	4.0680	5	C
A			
A	3.8980	5	A

The GLM Procedure

Duncan's Multiple Range Test for Chol

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.248158

Number of Means	**2**	**3**	**4**	**5**
Critical Range	.6572	.6898	.7106	.7251

Means with the same letter are not significantly different.

Duncan Grouping	**Mean**	**N**	**treat**
A	4.3620	5	E
A			
A	4.2360	5	B
A			
A	4.1900	5	D
A			
A	4.0680	5	C
A			
A	3.8980	5	A

The GLM Procedure

Tukey's Studentized Range (HSD) Test for Chol

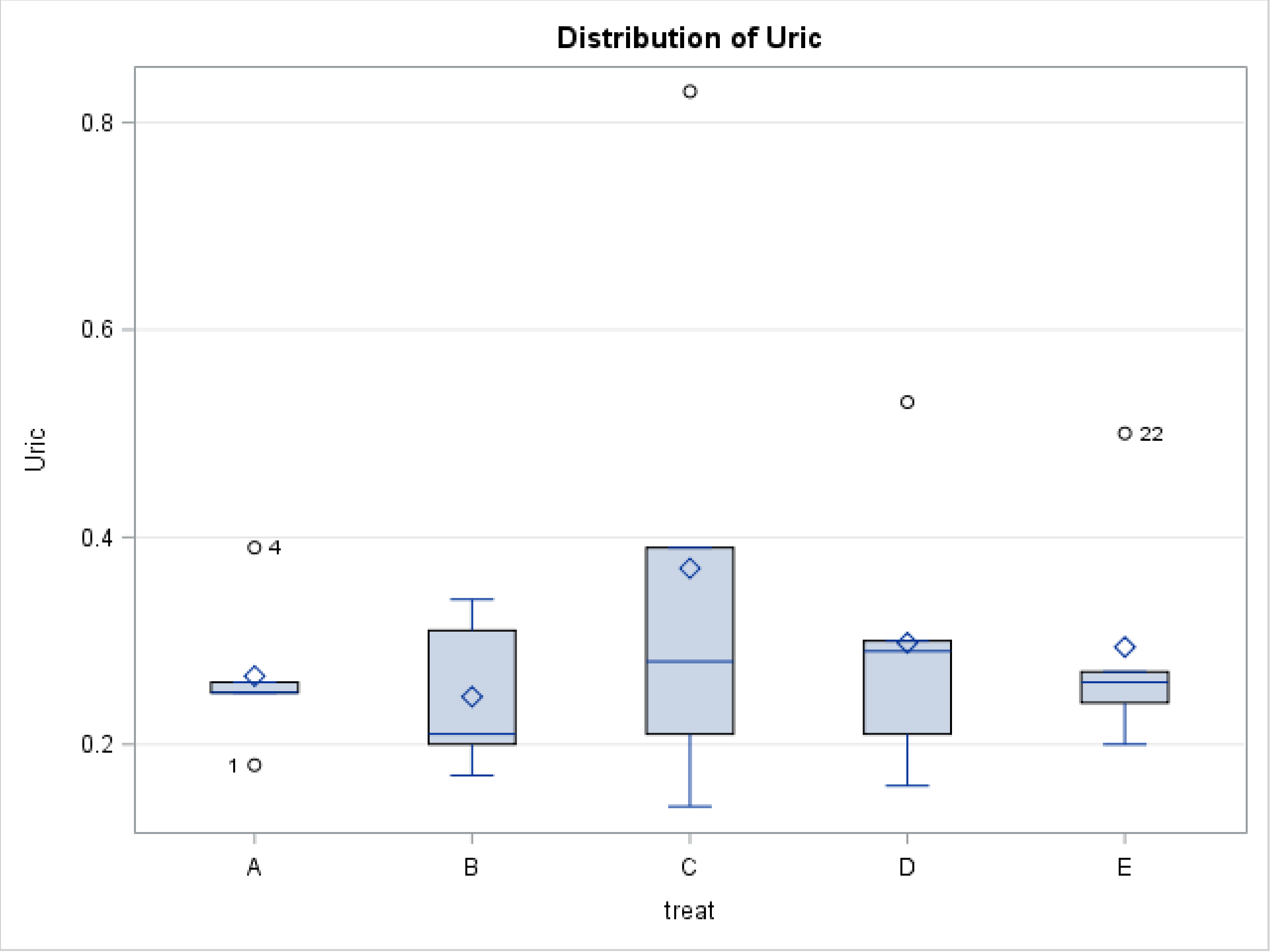
Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.248158
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.9428

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	4.3620	5	E
A			
A	4.2360	5	B
A			
A	4.1900	5	D
A			
A	4.0680	5	C
A			
A	3.8980	5	A

The GLM Procedure



The GLM Procedure

t Tests (LSD) for Uric

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.024032
Critical Value of t	2.08596
Least Significant Difference	0.2045

Means with the same letter are not significantly different.

t Grouping	Mean	N	treat
A	0.37000	5	C
A			
A	0.29800	5	D
A			
A	0.29400	5	E
A			
A	0.26600	5	A
A			
A	0.24600	5	B

The SAS System

The GLM Procedure

Duncan's Multiple Range Test for Uric

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.024032

Number of Means	**2**	**3**	**4**	**5**
Critical Range	.2045	.2147	.2211	.2256

Means with the same letter are not significantly different.

Duncan Grouping	**Mean**	**N**	**treat**
A	0.37000	5	C
A			
A	0.29800	5	D
A			
A	0.29400	5	E
A			
A	0.26600	5	A
A			
A	0.24600	5	B

The GLM Procedure

Tukey's Studentized Range (HSD) Test for Uric

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

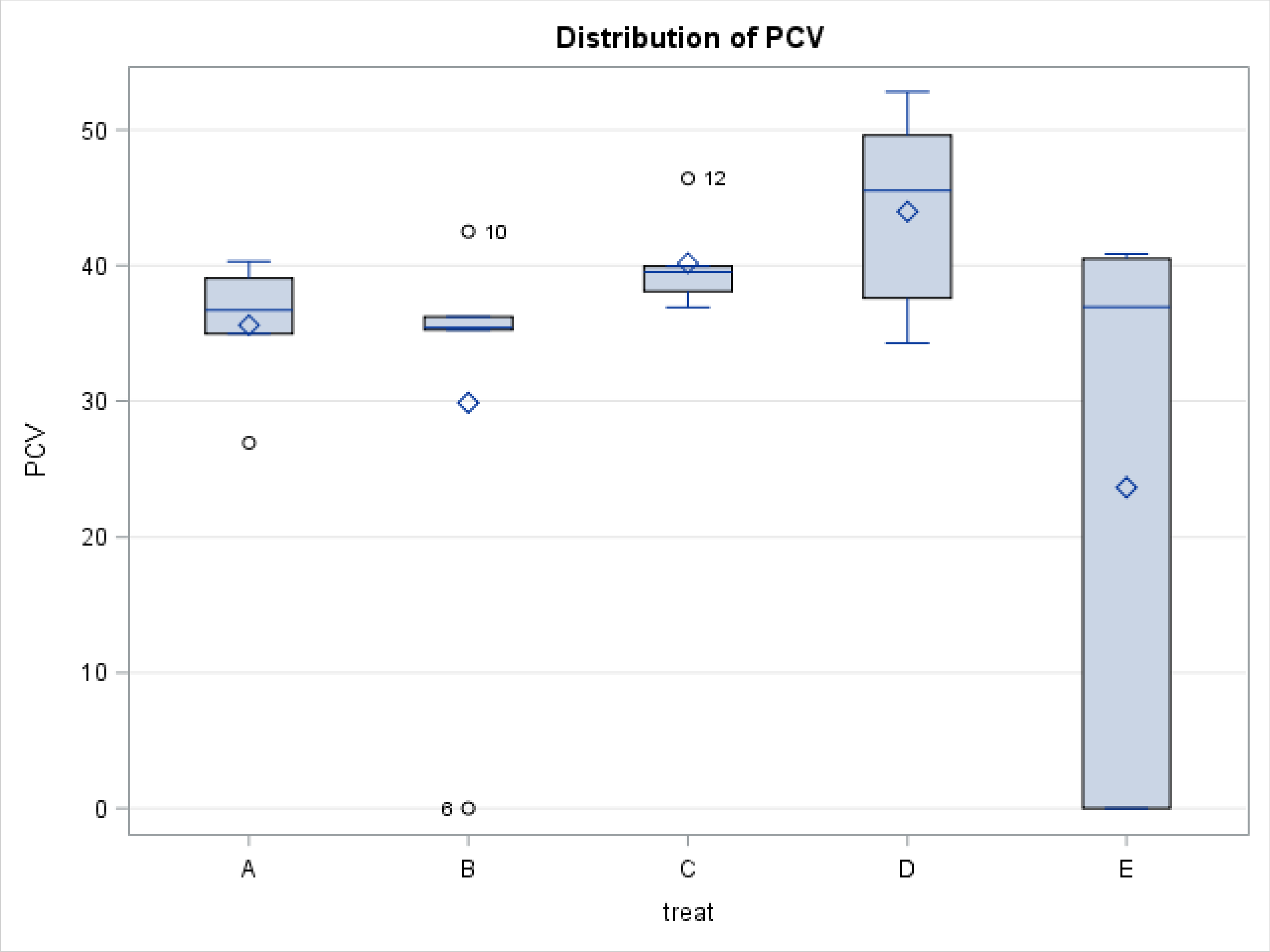
Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	0.024032
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	0.2934

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	treat
A	0.37000	5	C
A			
A	0.29800	5	D
A			
A	0.29400	5	E
A			
A	0.26600	5	A
A			
A	0.24600	5	B

The SAS System

The GLM Procedure



The GLM Procedure

t Tests (LSD) for PCV

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	171.9111
Critical Value of t	2.08596
Least Significant Difference	17.298

Means with the same letter are not significantly different.

t Grouping		Mean	N	treat
	A	43.950	5	D
	A			
B	A	40.176	5	C
B	A			
B	A	35.586	5	A
B	A			
B	A	29.866	5	B
B				
B		23.656	5	E

The GLM Procedure

Duncan's Multiple Range Test for PCV

Note: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	171.9111

Number of Means	**2**	**3**	**4**	**5**
Critical Range	17.30	18.16	18.70	19.08

Means with the same letter are not significantly different.

Duncan Grouping		**Mean**	**N**	**treat**
	A	43.950	5	D
	A			
B	A	40.176	5	C
B	A			
B	A	35.586	5	A
B	A			
B	A	29.866	5	B
B				
B		23.656	5	E

The SAS System

The GLM Procedure

Tukey's Studentized Range (HSD) Test for PCV

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	20
Error Mean Square	171.9111
Critical Value of Studentized Range	4.23186
Minimum Significant Difference	24.814

Means with the same letter are not significantly different.

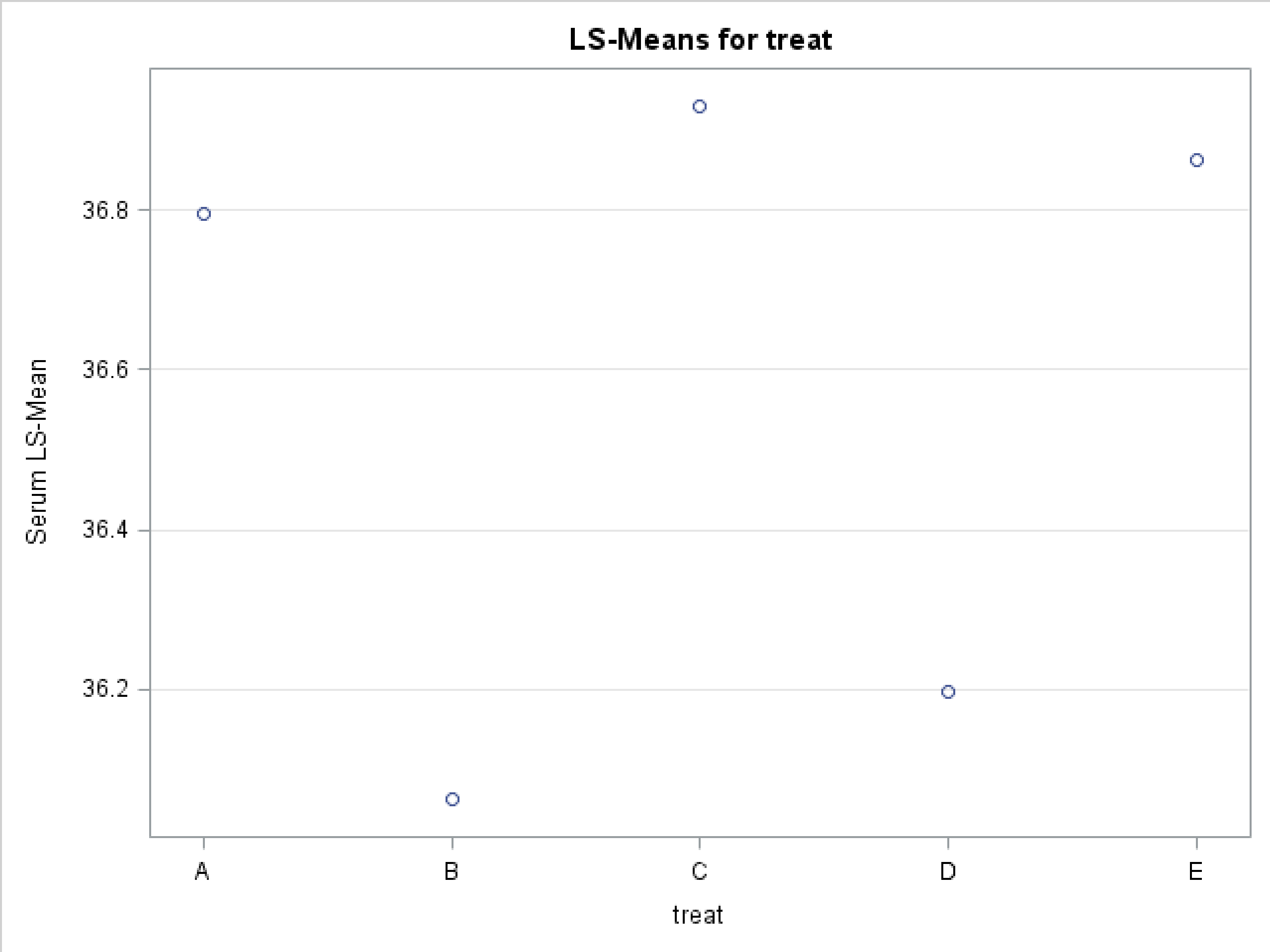
Tukey Grouping	Mean	N	treat
A	43.950	5	D
A			
A	40.176	5	C
A			
A	35.586	5	A
A			
A	29.866	5	B
A			
A	23.656	5	E

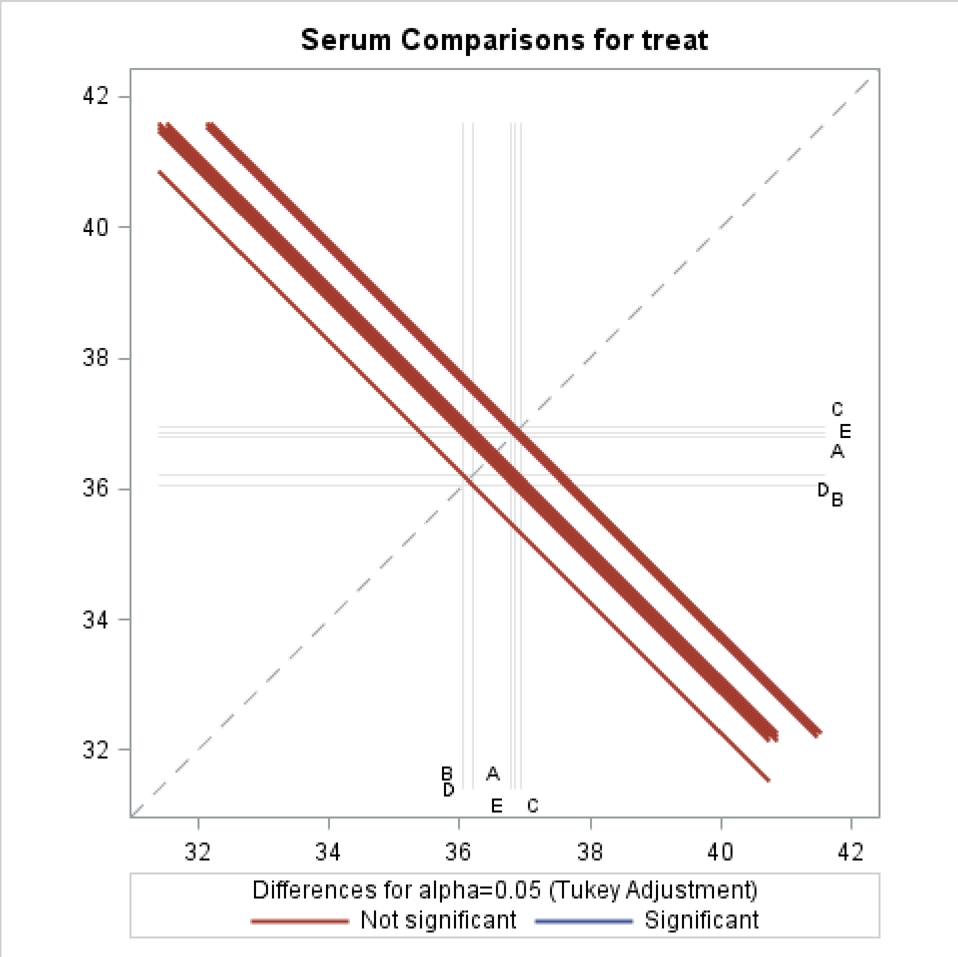
The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

treat	Serum LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	36.7960000	2.2060657	<.0001	1
B	36.0620000	2.2060657	<.0001	2
C	36.9300000	2.2060657	<.0001	3
D	36.1960000	2.2060657	<.0001	4
E	36.8620000	2.2060657	<.0001	5

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Serum

i/j	1	2	3	4	5
1		0.9993	1.0000	0.9997	1.0000
2	0.9993		0.9986	1.0000	0.9990
3	1.0000	0.9986		0.9993	1.0000
4	0.9997	1.0000	0.9993		0.9995
5	1.0000	0.9990	1.0000	0.9995	


LS-Means for treat
Serum LS-Mean
36.8
36.6
36.4
36.2
A
B
C
D
E
treat



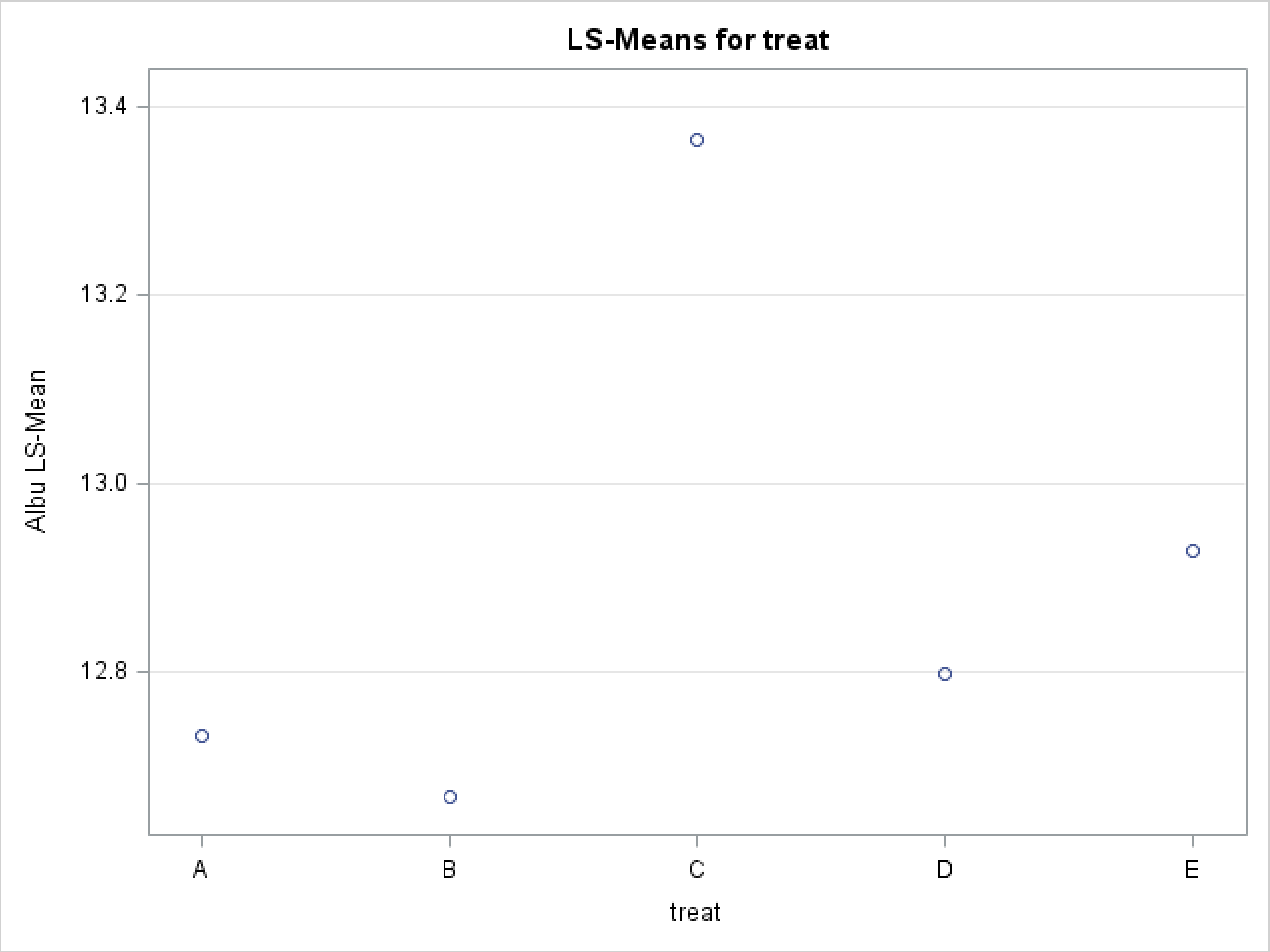
treat	Albu LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	12.7320000	0.6719202	<.0001	1
B	12.6660000	0.6719202	<.0001	2
C	13.3640000	0.6719202	<.0001	3
D	12.7980000	0.6719202	<.0001	4
E	12.9280000	0.6719202	<.0001	5

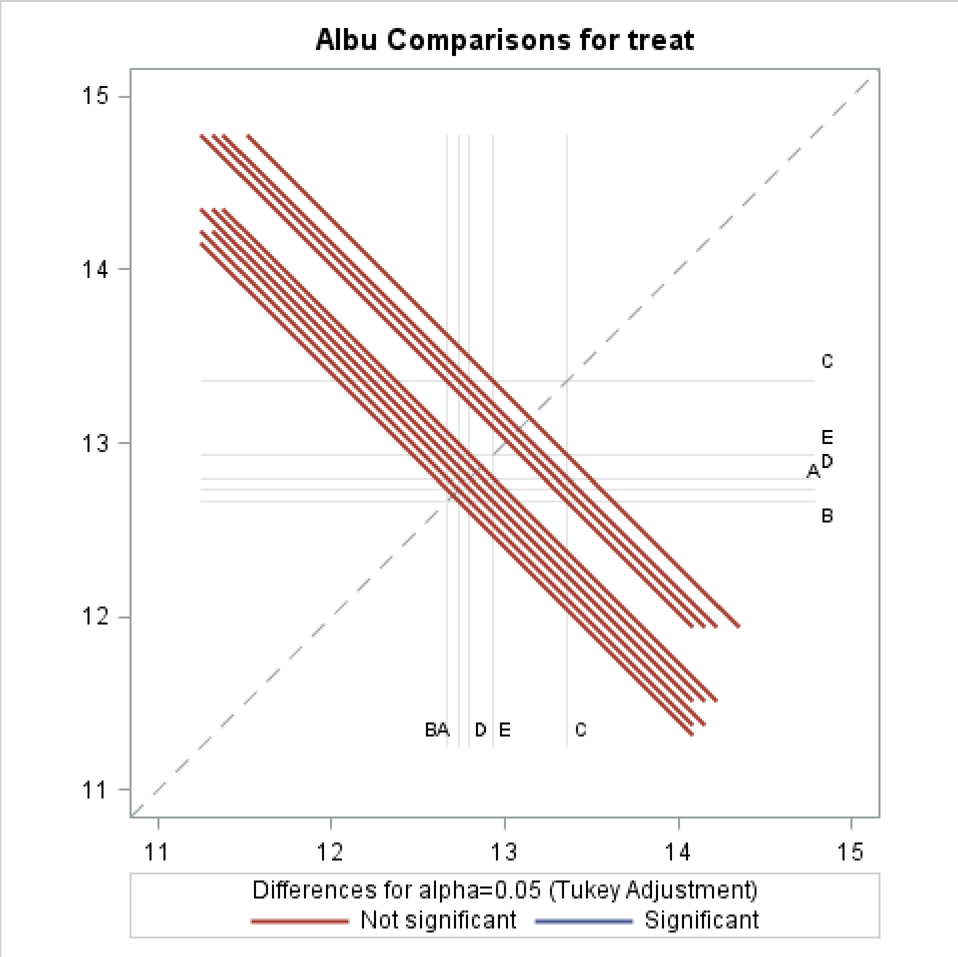
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Albu

i/j	1	2	3	4	5
1		1.0000	0.9616	1.0000	0.9996
2	1.0000		0.9458	0.9999	0.9986
3	0.9616	0.9458		0.9742	0.9902
4	1.0000	0.9999	0.9742		0.9999

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Albu

i/j	1	2	3	4	5
5	0.9996	0.9986	0.9902	0.9999	





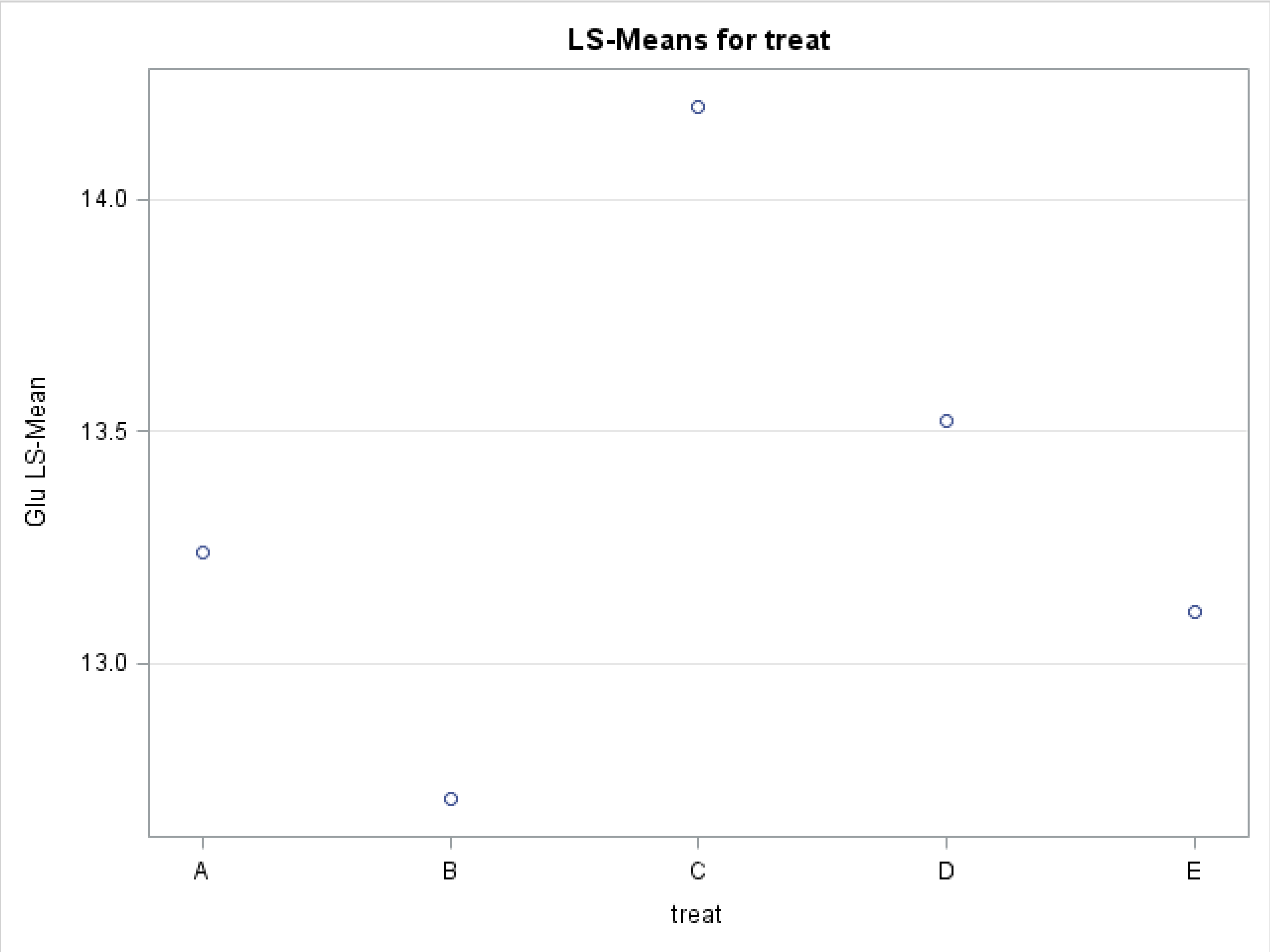
treat	Glu LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	13.2400000	0.4052579	<.0001	1
B	12.7060000	0.4052579	<.0001	2
C	14.2000000	0.4052579	<.0001	3
D	13.5200000	0.4052579	<.0001	4
E	13.1080000	0.4052579	<.0001	5

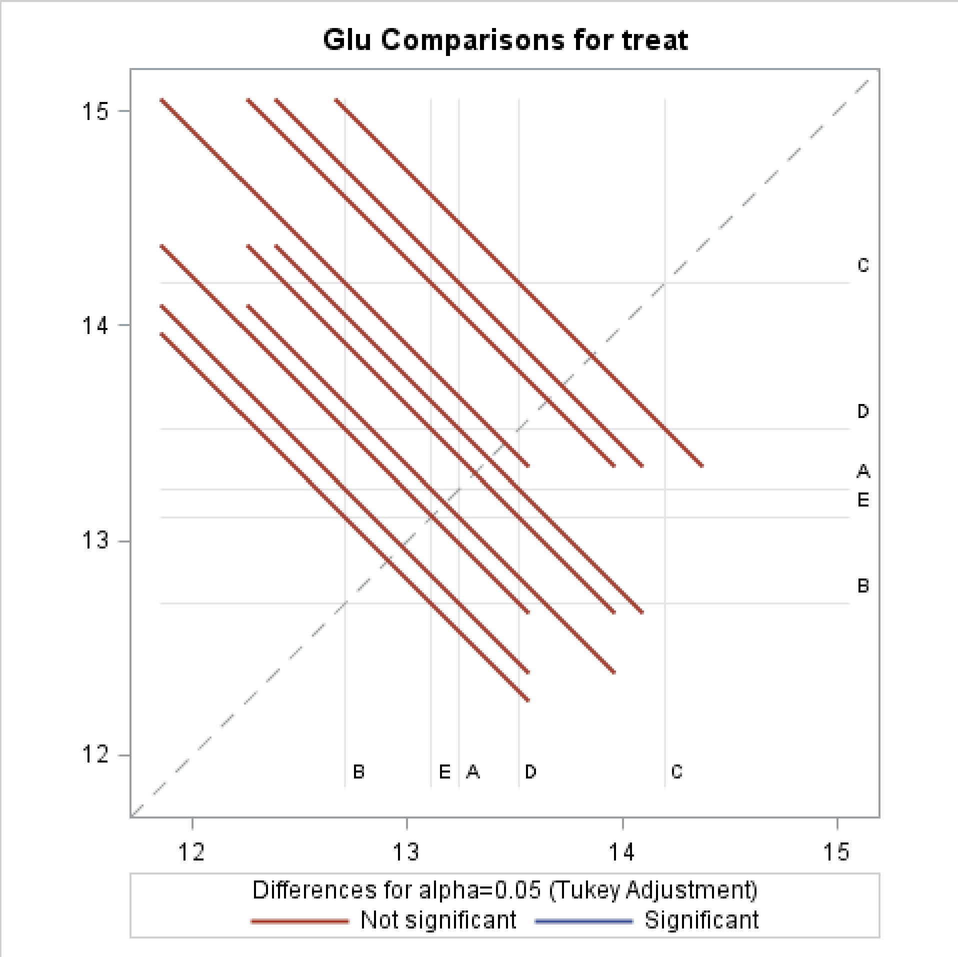
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Glu

i/j	1	2	3	4	5
1		0.8812	0.4704	0.9876	0.9993
2	0.8812		0.1069	0.6224	0.9538
3	0.4704	0.1069		0.7588	0.3465
4	0.9876	0.6224	0.7588		0.9497

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Glu

i/j	1	2	3	4	5
5	0.9993	0.9538	0.3465	0.9497	





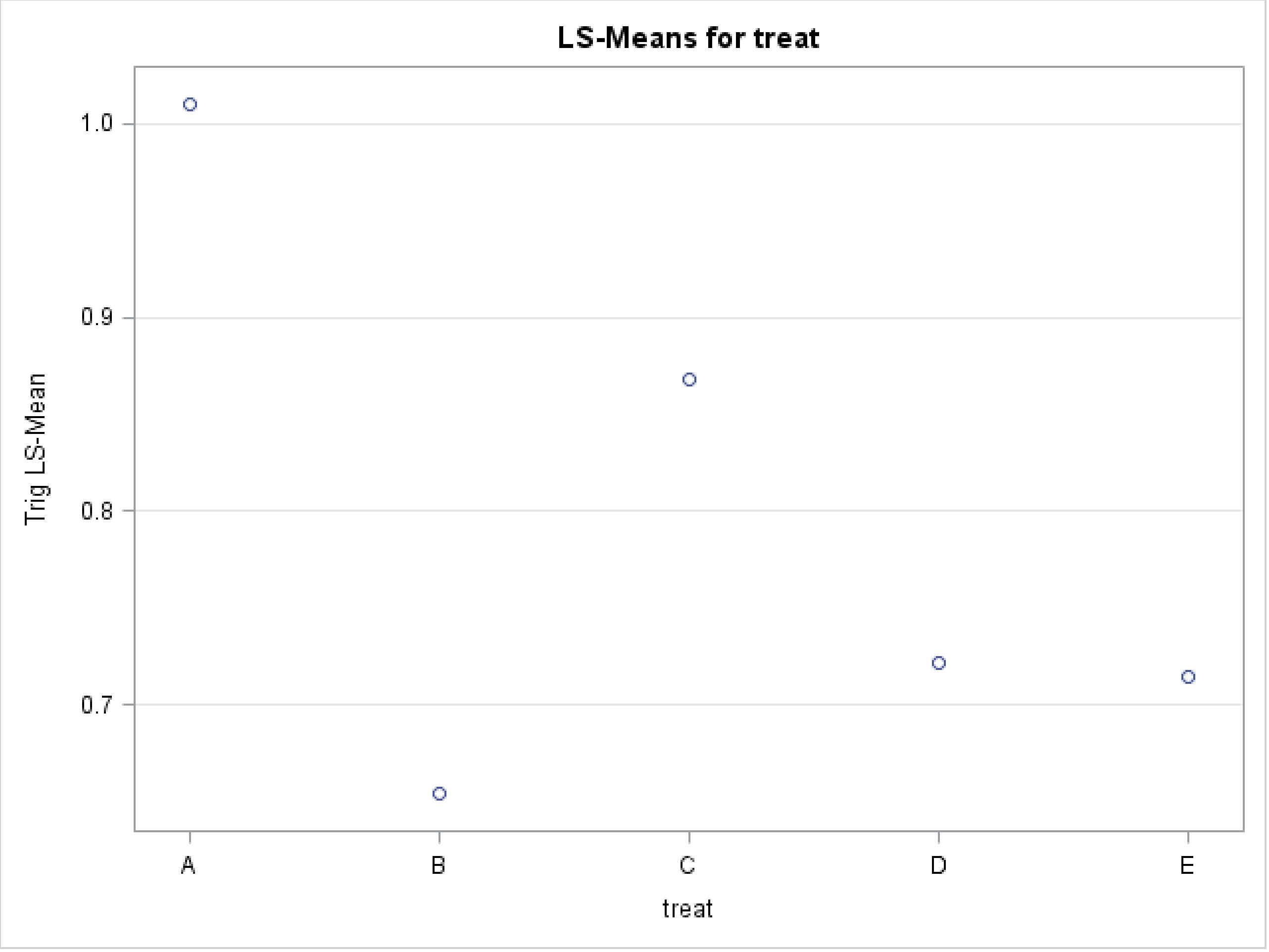
treat	Trig LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	1.01000000	0.20859051	<.0001	1
B	0.65400000	0.20859051	0.0052	2
C	0.86800000	0.20859051	0.0005	3
D	0.72200000	0.20859051	0.0025	4
E	0.71400000	0.20859051	0.0027	5

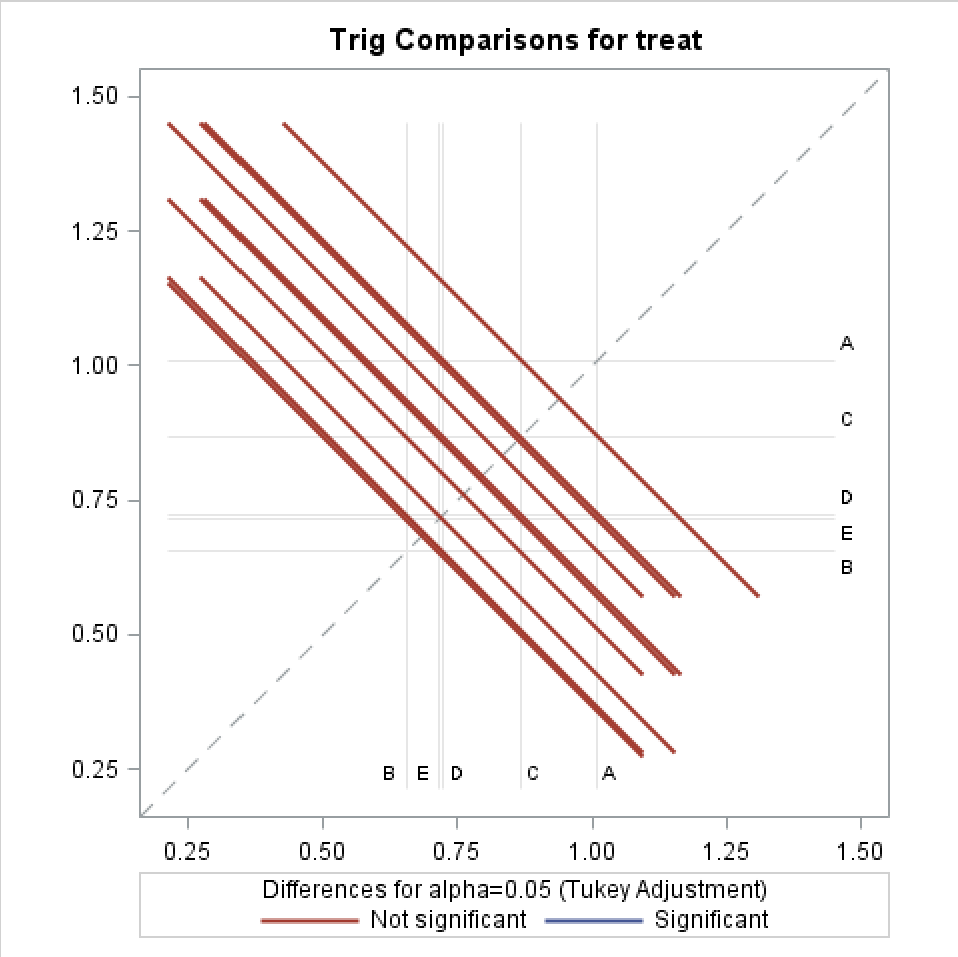
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Trig

i/j	1	2	3	4	5
1		0.7476	0.9882	0.8627	0.8507
2	0.7476		0.9480	0.9993	0.9996
3	0.9882	0.9480		0.9869	0.9841
4	0.8627	0.9993	0.9869		1.0000

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Trig

i/j	1	2	3	4	5
5	0.8507	0.9996	0.9841	1.0000	





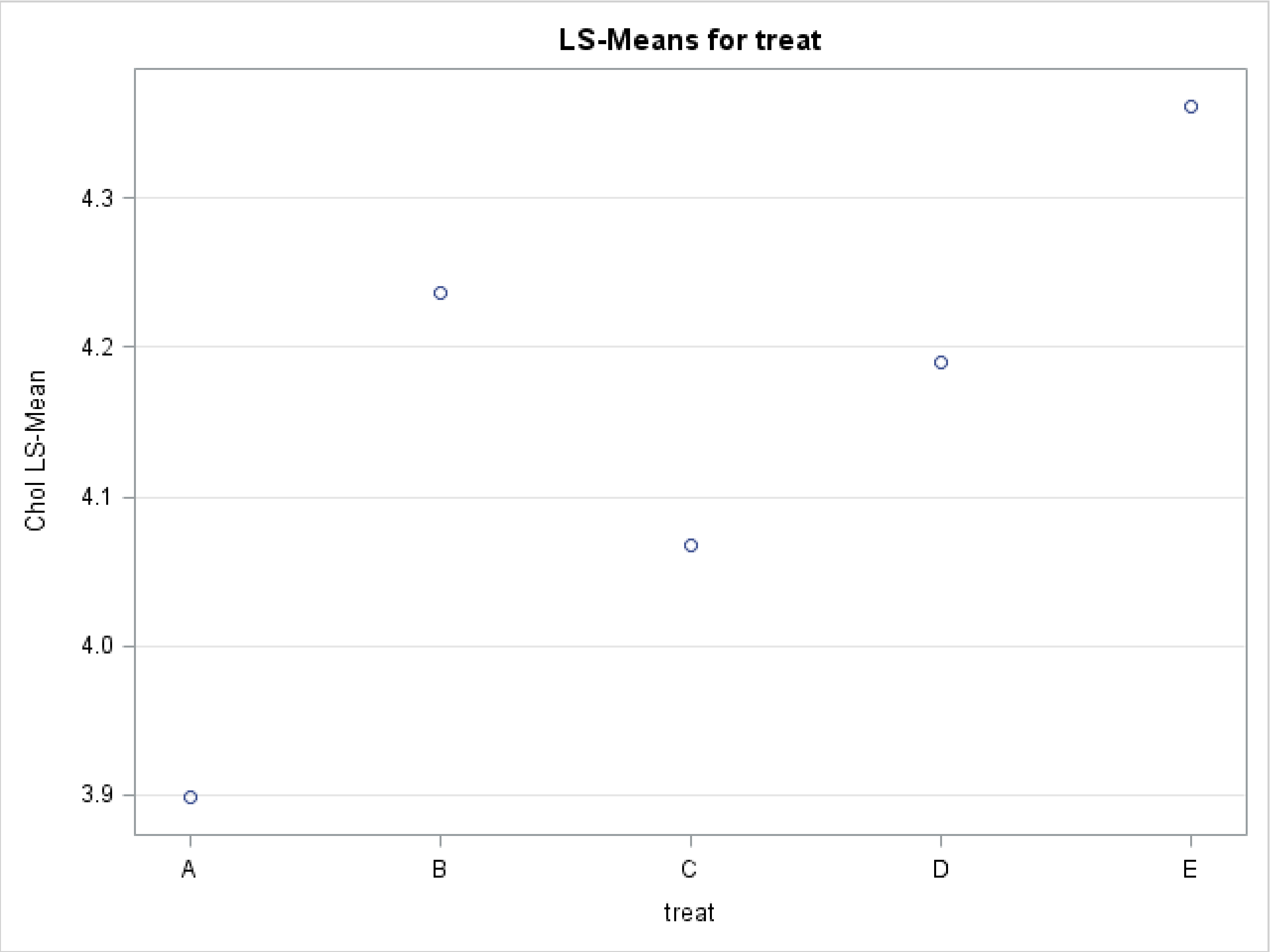
treat	Chol LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	3.89800000	0.22278151	<.0001	1
B	4.23600000	0.22278151	<.0001	2
C	4.06800000	0.22278151	<.0001	3
D	4.19000000	0.22278151	<.0001	4
E	4.36200000	0.22278151	<.0001	5

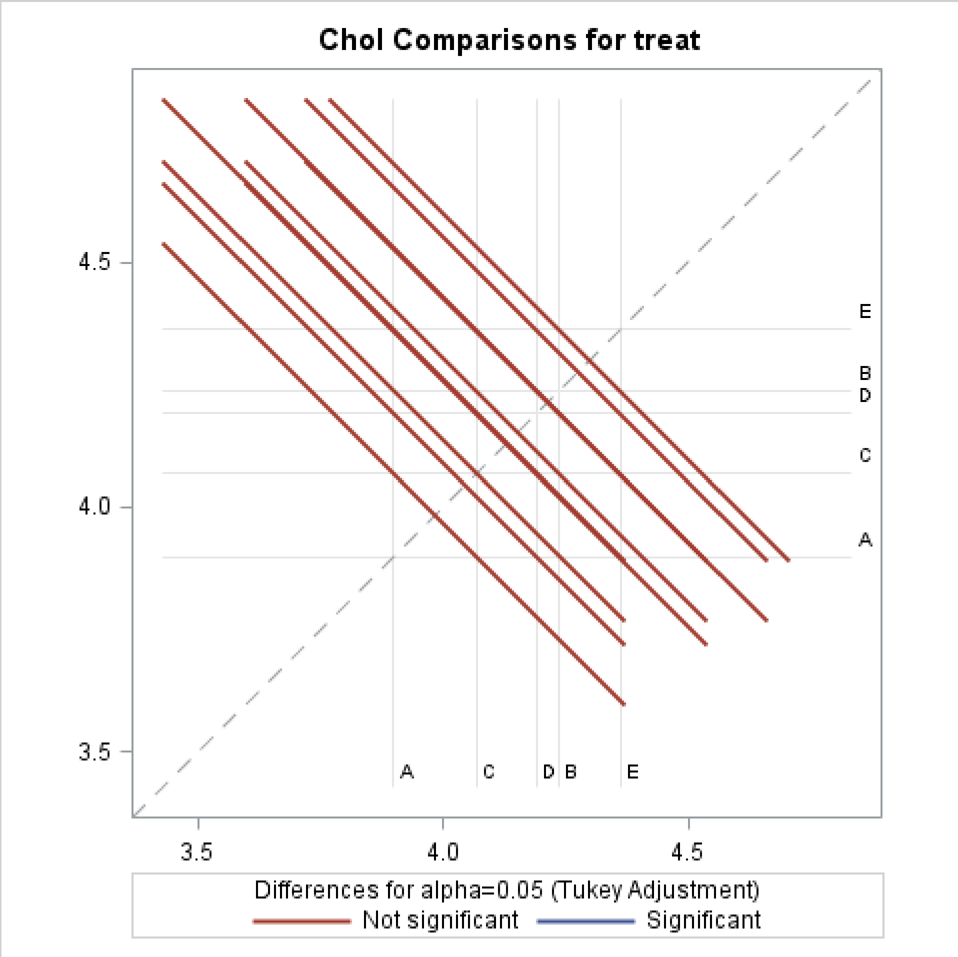
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Chol

i/j	1	2	3	4	5
1		0.8181	0.9820	0.8831	0.5907
2	0.8181		0.9828	0.9999	0.9942
3	0.9820	0.9828		0.9948	0.8806
4	0.8831	0.9999	0.9948		0.9812

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Chol

i/j	**1**	**2**	**3**	**4**	**5**
5	0.5907	0.9942	0.8806	0.9812	





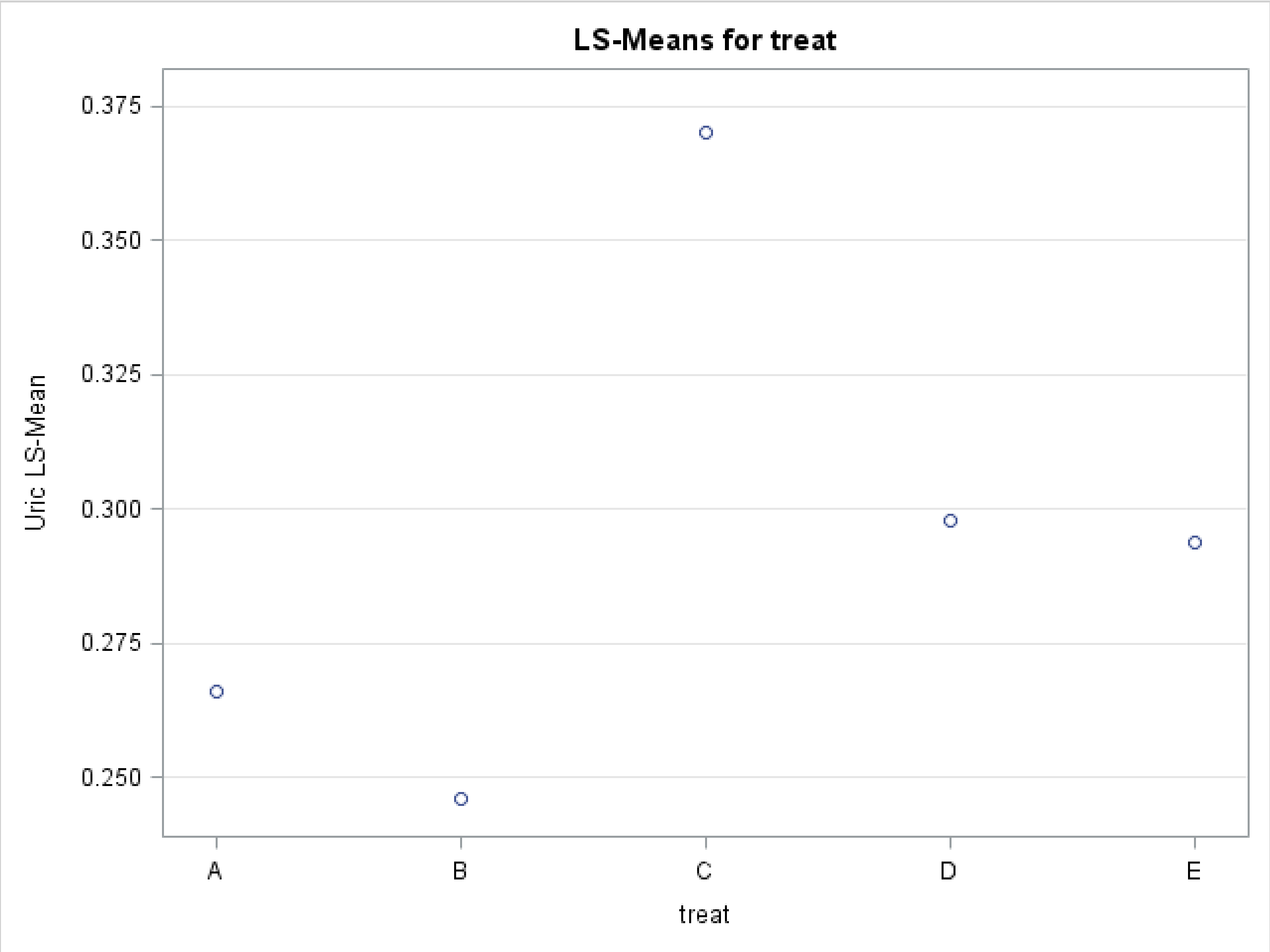
treat	Uric LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	0.26600000	0.06932820	0.0010	1
B	0.24600000	0.06932820	0.0020	2
C	0.37000000	0.06932820	<.0001	3
D	0.29800000	0.06932820	0.0004	4
E	0.29400000	0.06932820	0.0004	5

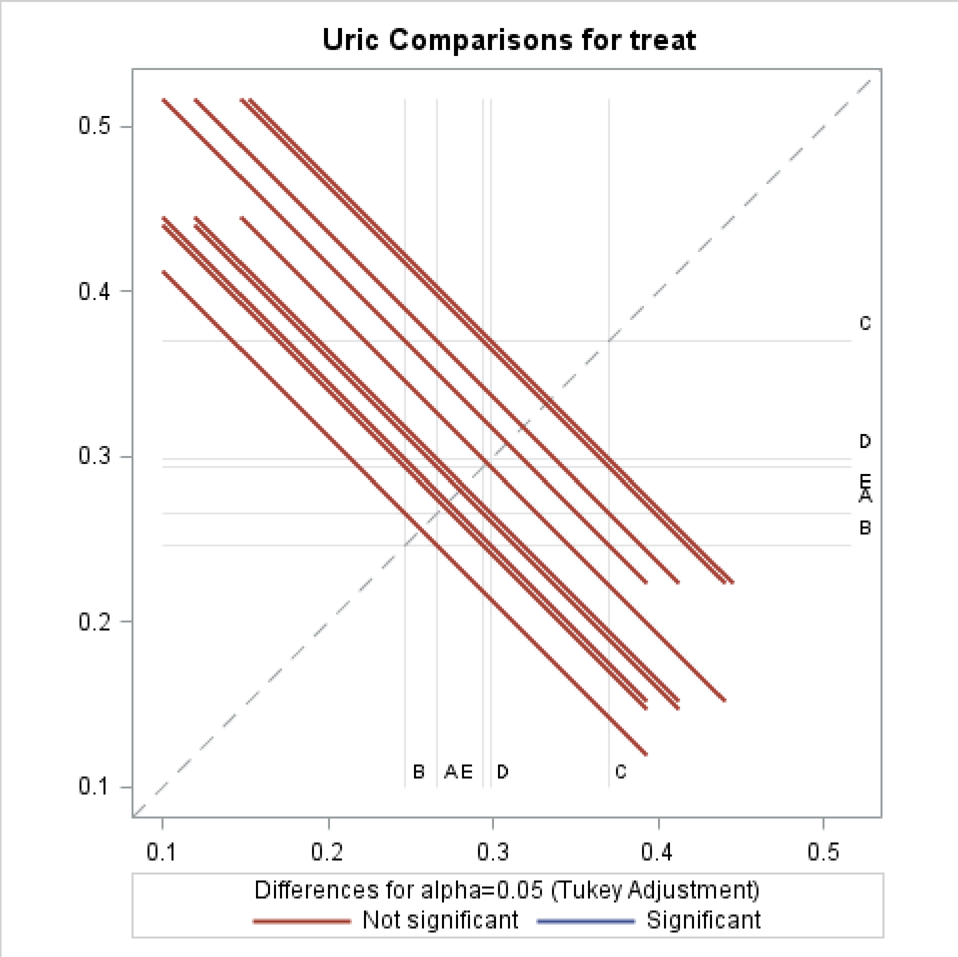
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Uric

i/j	1	2	3	4	5
1		0.9996	0.8240	0.9973	0.9984
2	0.9996		0.7147	0.9831	0.9875
3	0.8240	0.7147		0.9458	0.9348
4	0.9973	0.9831	0.9458		1.0000

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: Uric

i/j	1	2	3	4	5
5	0.9984	0.9875	0.9348	1.0000	





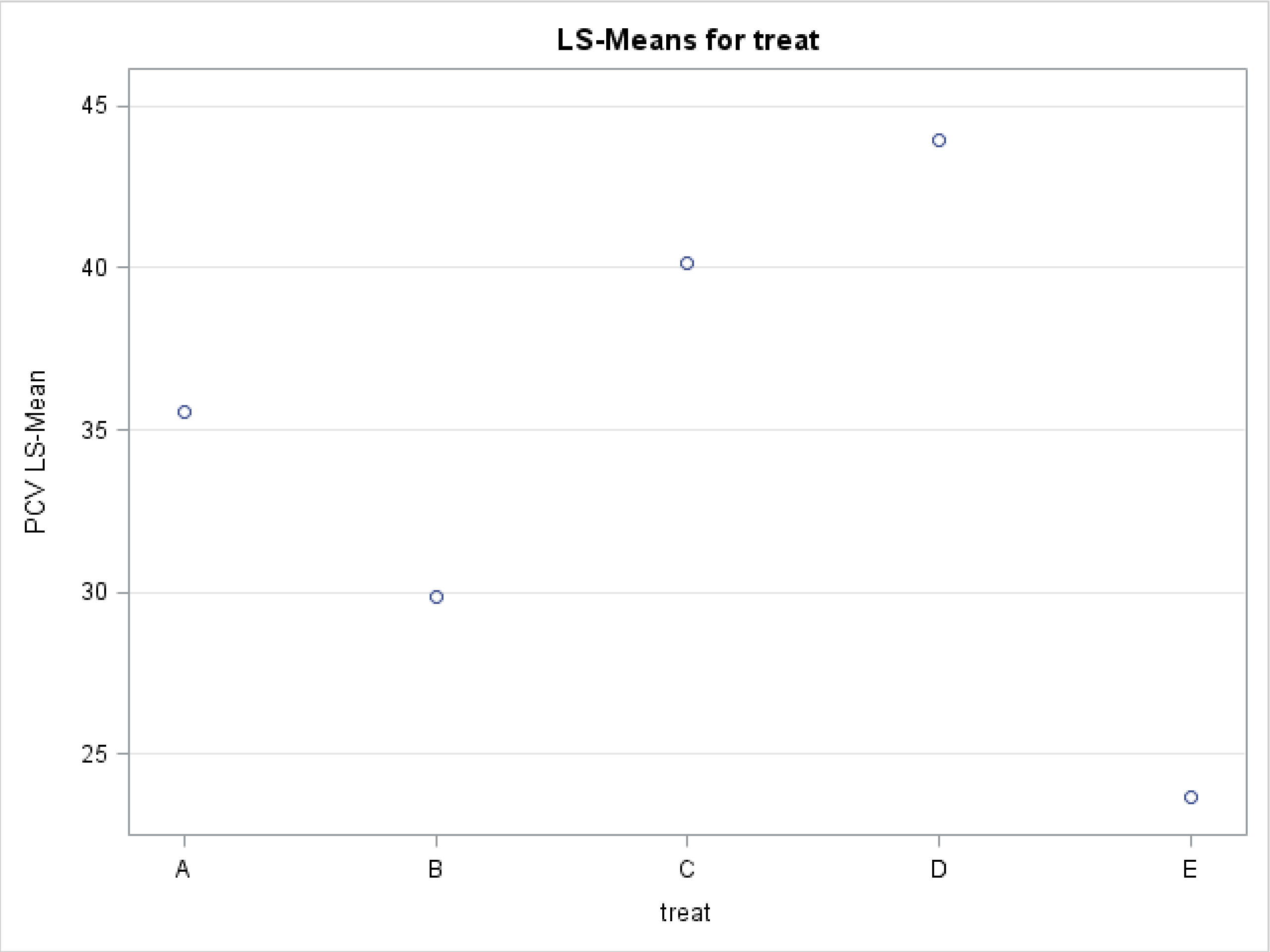
treat	PCV LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	35.5860000	5.8636361	<.0001	1
B	29.8660000	5.8636361	<.0001	2
C	40.1760000	5.8636361	<.0001	3
D	43.9500000	5.8636361	<.0001	4
E	23.6560000	5.8636361	0.0006	5

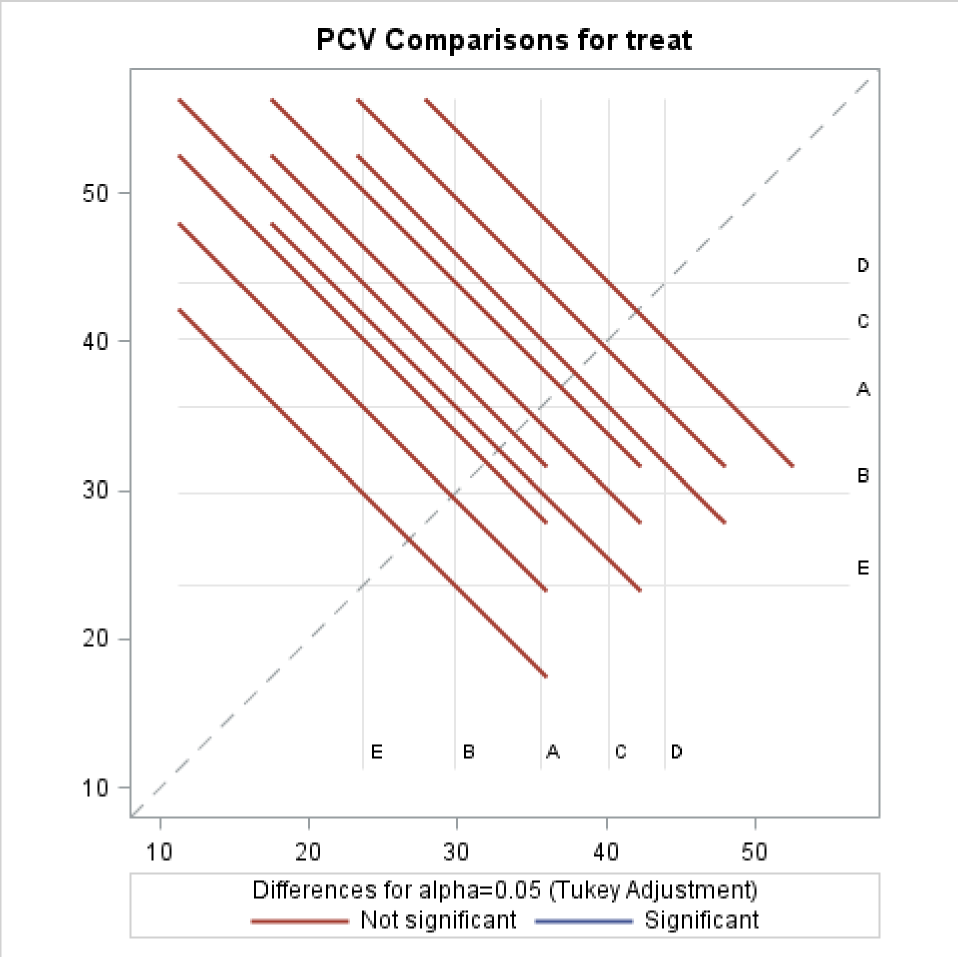
Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: PCV

i/j	1	2	3	4	5
1		0.9564	0.9802	0.8484	0.6113
2	0.9564		0.7270	0.4570	0.9421
3	0.9802	0.7270		0.9905	0.3052
4	0.8484	0.4570	0.9905		0.1435

Least Squares Means for effect treat
Pr > |t| for H0: LSMean(i)=LSMean(j)
Dependent Variable: PCV

i/j	1	2	3	4	5
5	0.6113	0.9421	0.3052	0.1435	





PCV Comparisons for treat
50
40
30
20
10
D
C
A
B
E
E
B
A
C
D
10
20
30
40
50
Differences for alpha=0.05 (Tukey Adjustment)
Not significant
Significant